University School of Medicine of Napoli Federico II

Department of Neuroscience

Director Prof. Lucio Annunziato

Differential impact of N-Methyl-D-Aspartate Receptor antagonists on genes involved in synaptic plasticity and neural glucose metabolism: implication for psychosis

Tutor

Prof. Andrea de Bartolomeis

Ph.D. Student

Dr Chiara Sarappa

XXIV course

Chapter 1.

Introduction.

- Schizophrenia psychopathology.
- The Dopaminergic Hypothesis of Psychosis.
- Glutamatergic Mechanisms of Psychosis: relevance for preclinical Animal Model of Psychosis.
- The N-Methyl-D-Aspartate Receptor (NMDA-R) Hypofunction Hypothesis of Psychosis.
- The Dopamine- Glutamate Interplay in Psychosis and the Post-Synaptic Density (PSD).
- PSD members and Immediate Early genes (IEGs).
- Glutamatergic mechanisms of antipsychotic drugs and new antipsychotic compounds with glutamatergic action.
- Aims of the research.

Chapter 2. Experimental procedures.

Chapter 3. Modulation of Hexokinase 1 and Glucose Transporter 3 in an animal model of psychosis: a putative link between metabolism and behavioral disorders.

Chapter 4. Modulation of Dopamine Receptors type 1-2 and Dopamine Transporter in an animal model of psychosis: acute and subchronic paradigms.

Chapter 5. Modulation of constitutive and inducible early genes implicated in glutamate neurotransmission by different antagonists at NMDA receptors.

Chapter 6. Discussion and Conclusion

References

Chapter 1.

Introduction.

Schizophrenia psychopathology.

Schizophrenia is a chronic, severe, and disabling mental disorder, with a global lifetime prevalence of about 0.3–0.7%. Schizophrenia is characterized by a disintegration of thought processes and of emotional responsiveness. Symptoms are classified in positive symptoms, such as auditory hallucinations, paranoid or bizarre delusions, and movement disorders (agitation or catatonia); in negative and cognitive symptoms such as blunted affect, alogia, anhedonia (inability to experience pleasure), working memory impairment, significant social withdrawal and vocational dysfunction. The onset of symptoms typically occurs in young adulthood (20–28 years for males and 26–32 years for females), with a pre-onset phase of the illness, characterized by transient or self-limiting psychotic symptoms and the non-specific symptoms of social withdrawal, irritability, dysphoria. In patients with a first episode of psychosis a good long term outcome occurs in about 42%, an intermediate outcome in 35% and a poor outcome in 27%.

The Dopaminergic Hypothesis of Psychosis.

Dysfunctions in dopamine and glutamate neurotransmissions, as well as in their interplay, have been suggested in the pathophysiology of psychosis (de Bartolomeis et al. 2005).

In its more traditional postulation, the dopaminergic hypothesis of psychosis states that putative subcortical hyperdopaminergy, stemming from overactivation of meso-striatal pathways, may cause productive psychotic symptoms (Kapur 2004).

Several lines of evidence confirm the involvement of the dopamine system in the pathophysiology and therapy of psychosis and seem to corroborate the dopaminergic hypothesis of psychosis: 1) dopamine-agonists, such as amphetamine, exerts a psychotomimetic action; amphetamine increases striatal dopamine levels, triggers psychotic symptoms or worsens psychotic symptoms in schizophrenic patients (Lieberman et al. 1987; Howes and Kapur 2009); 2) schizophrenic subjects exhibit increased striatal release of dopamine after injection of amphetamine compared to healthy volunteers as demonstrated by in vivo positron emission tomography (Breier et al. 1997); 3) the most effective compounds against psychotic symptoms, the so-called antipsychotic agents, used since 1952 (Delay et al. 1952), share dopamine D2 receptor (D2R) antagonist properties (Snyder 1976) and increase the dopamine metabolism in rat brain (Carlsson and Lindqvist 1963); 4) therapeutic efficacy of antipsychotics is tightly correlated to the degree of affinity and blockade of dopamine D2Rs (Creese et al. 1976).

A subsequent refinement of the dopaminergic hypothesis of psychosis has hypothesized that a decreased function of the dopaminergic neurons belonging to the meso-cortico-limbic tract may explain the cognitive dysfunctions that are observed in schizophrenia. This view has been called the “cortical hypodopaminergia hypothesis” (Kapur 2004) and has been suggested to explain.

The dopaminergic hypothesis ascribes positive symptoms of schizophrenia (suspiciousness, persecution and grandiosity delusions and hallucination) to presynaptic striatal hyperdopaminergia D2R mediated, and negative (depression, flattened affect, social withdrawal) and cognitive symptoms (cognitive impairment, disorganized thinking, autistic behaviors) to prefrontal hypodopaminergia D1-receptor (D1R) mediated (Pycock et al. 1980; Scatton et al. 1982; Carlsson 1988; Davis et al. 1991; Abi-Dargham et al. 2002; Davidson and Heinrichs 2003; Goldman-Rakic et al. 2004; Tamminga 2006; Howes and Kapur 2009; Javitt 2010). Preliminary data in preclinical settings have been confirmed by PET and SPECT studies that have observed an increase in blood flow in the striatum (Laruelle et al. 1996; Breier et al. 1997; Abi-Dargham et al. 1998) and a decrease in blood flow in cortical areas of psychotic patients (Erritzoe et al. 2003).

Glutamatergic Mechanisms of Psychosis: relevance for preclinical Animal Model of Psychosis.

Since 1950s, Phencyclidine (PCP) has been observed to exacerbate psychotic symptoms in schizophrenic individuals and to induce a schizophrenia-like state in healthy subjects characterized by hostility, agitation, auditory hallucinations, and paranoic delusions (Allen and Young 1978). In 1980s, PCP was characterized as a non-competitive antagonist of the glutamate N-Methyl-D-Aspartate-Receptor (NMDA-R) (Lodge and Anis 1982; Javitt 1987; Javitt and Zukin 1991); implying the possibility that glutamatergic neurotransmission also may be implicated in the pathophysiology of psychosis. In 1990s, another non-competitive NMDA-R antagonist, ketamine, was described to induce in healthy volunteers a psychotic-state mimicking positive, negative and cognitive symptoms of schizophrenia (Javitt and Zukin 1991; Krystal et al. 1994; Adler et al. 1999; Heresco-Levy 2003; Krystal et al. 2003; Javitt 2010) and to exacerbate psychotic symptoms in both drug-free schizophrenic patients (Malhotra et al. 1997; Lahti et al. 2001; Heresco-Levy 2003) and in remitted patients (Lahti et al. 1995; Heresco-Levy 2003). The occurrence of negative symptoms in non-schizophrenic individuals had not been observed in other models of schizophrenia, rendering glutamatergic drugs-induced psychosis the best pharmacological model of disease in all its psychopathological manifestations.

Concomitant with clinical reports that non-competitive antagonists at NMDA-R may induce a schizophrenia-like psychosis, several animal studies were carried out to evaluate neuropathological and behavioral abnormalities in rats exposed to selective antagonists at NMDA-R (Carlsson and Svensson 1990; Tiedtke et al. 1990; Riederer et al. 1991; Schmidt et al. 1991). Neurodegenerative changes in corticolimbic regions of rat brains were associated with competitive or non-competitive blockade of NMDA-R

(Olney and Farber 1995). An acute vacuole reaction in neurons of the posterior cingulated and retrosplenial cortices was observed after a single low subcutaneous dose. Higher doses caused an extensive neuron-necrotizing reaction spreading to neurons in several additional neocortical and limbic brain regions (Allen and Iversen 1990; Olney et al. 1991; Sharp et al. 1994).

Acute sub-anaesthetic doses of non-competitive NMDA-R antagonists were found to induce several behaviors in rodents mimicking schizophrenia, including hyperlocomotion, enhanced stereotyped behaviors, cognitive and sensorimotor gating deficits, and impaired social interactions (Lipska and Weinberger 2000).

Thus, administration of non-competitive NMDA-R antagonists, as ketamine, may represent a powerful preclinical model of psychosis, with substantial heuristic properties to study both molecular targets in psychosis pathophysiology and novel therapeutic strategies (Lipska and Weinberger 2000).

The NMDA-R Hypofunction Hypothesis of Psychosis.

The histopathological, biochemical, and behavioral studies quoted above were the basis for the Olney and Farber (Olney et al. 1999) model of psychosis pathophysiology known as the NMDA-R hypofunction (NRH) hypothesis.

According to Olney & Farber hypothesis, hypofunction of NMDA-R may result in a decreased activation of GABAergic interneurons by collateral fibers of glutamatergic pyramidal neurons. Decreased activation of GABAergic interneurons result in a lack of inhibition of glutamatergic pyramidal neurons which increase their firing onto post-synaptic neurons located in other cortical regions, in the striatum and the mesencephalon. This has been suggested to lead to cortical neurotoxicity, while increased firing upon subcortical dopaminergic neurons may activate in turn over these.

NRH may thus lead to a complex cortical-subcortical network dysinhibition, involving several neurotransmitter systems (glutamatergic, cholinergic, serotoninergic, noradrenergic, GABAergic, dopaminergic), to a condition of excitotoxicity by hyper-glutamatergy upon non-NMDA-R (Moghaddam et al. 1997; Adams and Moghaddam 1998; Olney et al. 1999; Farber 2003; Krystal et al. 2003; Lisman et al. 2008) and to the dysinhibition of acetylcholine fibers from basal forebrain (Giovannini et al. 1994; Kim et al. 1999; Olney et al. 1999; Farber et al. 2002; Farber 2003). These biochemical dysfunctions may account for the histopathological and behavioral abnormalities seen in rodents.

The Dopamine- Glutamate Interplay in Psychosis and the Post-Synaptic Density.

Dopaminergic and glutamatergic systems are tightly interconnected in the forebrain at both the cellular and the subcellular level. The imbalance of the dopamine-glutamate interplay may represent one of the key mechanism in psychosis pathophysiology.

The prefrontal cortex (PFC) sends efferent glutamatergic projections to the nucleus accumbens and to other limbic areas (hippocampus and amygdale), which also modulate nucleus accumbens activity by glutamatergic projections (Grace 2007), and to dopaminergic neurons within ventrotegmental area (VTA) (Carr and Sesack 2000), which project to cortical and subcortical limbic areas. Dopamine neurons from the VTA and glutamate fibers from multiple cortical and subcortical areas, project to pyramidal glutamatergic neurons and GABA interneurons in the PFC.

This afferent-efferent network integrates sensory and limbic information and control cognitive and motor behaviors. PFC is assumed to exert a top-down control over incoming information and goal-directed behavior and to regulate cognitive and executive processes involving emotion, memory, motivation, and control of goal-directed behavior. The activation of NMDA-R on subcortical GABAergic neurons provides inhibitory control on excitatory glutamatergic thalamic fibers projecting to pyramidal neurons in the PFC. NRH on GABAergic neurons may cause a dysinhibition of glutamatergic thalamo-cortical neurons and may induce excessive stimulation of pyramidal neurons in the PFC. Indeed, PFC dysfunctions have consistently been described in schizophrenic patients (Krystal et al. 2003; Winterer and Weinberger 2004).

Moreover, dysfunctions of striatum have also been predicted to play a critical role in the pathophysiology of schizophrenia. Striatal neurons receive glutamatergic projections from prefrontal cortex, hippocampus, amygdala, and thalamus, belonging both to

sensory-motor and limbic systems; and dopaminergic projections from susbstantia nigra and VTA, constituting respectively the so-called meso-striatal and meso-limbic systems, which participate in the control of motor functions and in motivational aspects of behavior. Output projections from striatum are involved in the gating of information flow via thalamus to the prefrontal cortex.

Possible consequences of an NRH-induced dysinhibition of excitatory pathways may include the dysinhibition of dopamine meso-striatal release, that may explain positive symptoms; the inhibition of dopamine meso-cortical release on D1R that may explain negative symptoms; the inhibition of GABAergic inhibition on cortical-striatum-pallidum-thalamus-cortical network that may explain behavioral and cognitive disorders (Olney and Farber 1995; Moghaddam et al. 1997; Olney et al. 1999; Farber 2003). Hyperdopaminergia may *per se* cause hyperinhibition of glutamate release, thus worsening a condition of NRH (Olney et al. 1999). It has been proposed that aberrant changes at different levels (primary NRH or NRH equivalent condition, such as dopamine hyperfunction or GABAergic hyperhinhibition) may ultimately result in presynaptic dopamine dysregulation that may be the final common pathway to induce an "aberrant attribution of salience" to cortical inputs (Olney et al. 1999; Kapur and Mamo 2003; Howes and Kapur 2009; Javitt 2010). Aberrant salience has been attributed to the dysregulation in dopamine transmission and may be explained as an aberrant interpretation of external or internal events: delusions and hallucinations become the cognitive and perceptive explanation of these aberrant information (Kapur and Mamo 2003).

In striatum, dopamine and glutamate fibers take connections with GABAergic interneurons, called the medium-sized spiny neurons (MSNs). MSNs take part to the recurrent neuronal loop starting from the cortex and driving inputs back to the cortex via

basal ganglia and thalamus. Therefore, dopaminergic and glutamatergic inputs on these GABAergic neurons appear to regulate the output signal starting from MSNs. At the subcellular level, dopamine and glutamate interplay occurs within an electrondense thickening located at the post-synaptic sites of glutamatergic synapse (Okabe 2007; Sheng and Hoogenraad 2007), called the Post-Synaptic Density (PSD), which is enriched in the dendritic spines of MSNs. PSD is a complex protein network devoted to the intracellular integration of synaptic signals and to the fine-tuning of signal transduction. The PSD is a protein machinery that integrates synaptic signals from presynaptic neurons and different neurotransmitter systems, including the dopaminergic and the glutamatergic (de Bartolomeis et al. 2005). The PSD has been described as a protein mesh which allows physical bridging and functional connection between a wide array of intracellular molecules involved in synaptic signal transduction, including scaffold proteins, surface receptors, cytoskeletric factors, effectors of the second messengers pathways. This complex ultrastructure is in continuous re-modeling in response to synaptic signals. Stable or transient changes of PSD proteins have been suggested to be implicated in the pathophysiology of a number of neuropsychiatric diseases (de Bartolomeis and Iasevoli 2003; Szumlinski et al. 2006; Gardoni et al. 2009; Cheng et al. 2010). Moreover, we have observed that gene expression of PSD proteins may be modulated by antipsychotic drugs, implying that PSD may also be involved in the mechanism of action of these agents (Polese et al. 2002; de Bartolomeis and Iasevoli 2003; Iasevoli et al. 2007; Iasevoli et al. 2010; Tomasetti et al. 2011).

PSD members and Immediate Early genes (IEGs).

Homer.

Homer proteins are a family of constitutive and inducible scaffold proteins of glutamatergic synapses, involved in signal transduction, modulation of local calcium dynamics, and neuronal development. In humans, Homer proteins, codified by different genes on different chromosomes: Homer 1 (chromosome 5q14.2), Homer 2 (chromosome 15q24.3) and Homer 3 (chromosome 19p13.11), all of which exhibit numerous isoforms (Shiraishi-Yamaguchi and Furuichi 2007). Homer 1b/c (*H1b/c*), Homer 2a/b (*H2a/b*) and Homer 3a/b (*H3a/b*) are long Homer forms characterized by a conserved amino-terminal EVH1 domain that binds proline-rich sequences, and a carboxy-terminal coiled-coil domain that allows homo-multimerization. Homer proteins bind several targets within the PSD, including type I metabotropic receptors of glutamate (mGluR1α/5), the Inositol Trisphosphate-Receptor (IP3-R), ryanodine receptor, the NMDA-R scaffolding protein Shank, the transient receptor potential canonical-1 (TRPC-1), G-guanosine trisphosphatases (GGTPases), actin cytoskeleton, transcription factors such as NFTA and PAX6 (Szumlinski et al. 2006; Shiraishi-Yamaguchi and Furuichi 2007; Foa and Gasperini 2009).

In response to synaptic activity, Homer homomerics different target proteins in close proximity under the membrane, to facilitate the glutamate-mediate signal transduction and the cross-talking among different pathways; Homer declustering is induced by increasing of intracellular calcium through NMDA channels or voltage-dependent calcium channel (Szumlinski et al. 2006; Shiraishi-Yamaguchi and Furuichi 2007).

Short forms of Homer, Homer 1a (*H1a*), Ania 3 (*Ania3*), Homer 2c/d (*H2c/d*), Homer 3c/d (*H3c/d*) lack the carboxy-terminal coiled- coil domain, therefore they can not homo-multimerize. Once induced, these short isoforms may decouple long Homer-

mediated homo-hetero-clusters. *H1a* and *Ania3* behave as immediate early genes (IEGs), whose expression is induced by several neuronal stimuli, such as seizure, kindling, dopamine stimulation, long-term potentiation, psychoactive stimulants and drugs, exploration of novel environment, stimulation by light, sleep loss, learning activity (Bottai et al. 2002; Szumlinski et al. 2006; Shiraishi-Yamaguchi and Furuichi 2007).

In previous studies, it has been observed that the prototype "typical" antipsychotic haloperidol triggers the expression of the inducible Homer1 variant *Homer1a* (de Bartolomeis et al. 2002). *Homer1a* expression has also been found induced by atypical antipsychotics, although with divergent quantitative and topographic patterns when compared to haloperidol (Ambesi-Impiombato et al. 2007; Iasevoli et al. 2009; Iasevoli et al. 2010). Moreover, *Homer1a* expression is significantly induced by administration of a compound with relative selective antagonism at dopamine D2Rs (Iasevoli et al. 2009). Homers may thus represent adaptor molecules that facilitate cross-talk between the glutamatergic and dopaminergic systems and may be involved in the molecular mechanism by which dopaminergic compounds may modulate glutamatergic signaling.

Intriguingly, *Homer1* knock-out mice exhibited some schizophrenia-related phenotypes, including disrupted PPI, impaired working memory performance, increased locomotor response to MK-801 and amphetamine (Yuan et al. 2003; Szumlinski et al. 2005). These mice also showed increased levels of glutamate in the prefrontal cortex and decreased levels in the nucleus accumbens, consistent with the cortical hyperglutamatergic/striatal hypoglutamatergic state postulated in schizophrenia (Szumlinski et al. 2005). Moreover, a trend toward an association with schizophrenia has been described in a European population (Norton et al. 2003) and *Homer1a* mRNA

is overexpressed in the pharmacological animal model of psychosis provided by acute administration of subanaesthetic doses of ketamine (Iasevoli et al. 2007).

PSD95.

The Postsynaptic Density Protein 95 (PSD95), also known as Synapse-Associated Protein 90 (SAP-90), is a protein encoded by the *DLG4* (disks large homolog 4) gene (Cho et al. 1992; Hunt et al. 1996; Stathakis et al. 1997). PSD95 is a member of the membrane- associated guanylate kinase (MAGUK) family. PSD95 has three PDZ C-terminal domains, a SH3 domain and a guanylate kinase domain, but it lacks of ATP binding site, being catalytically inactive (Woods and Bryant 1993). It heteromultimerizes with PSD93. Recruiting this protein into the same postsynaptic sites to form a multimeric scaffold for the clustering of receptors, such as NMDA-R and AMPA-R, a Calcium-activated form of nitric oxide synthase, ion channels, such as potassium channel, and associated signaling proteins such as neuroligin and calmoduline (Hunt et al. 1996). Regulatory processes, such as phosphorylation, modify PSD95 and its ligand to determinate assembly or disassembly of macromolecular complexes (Gardoni et al. 2009). PSD95 regulates dendritic spine size and shape (Sala et al. 2005). Therefore, PSD95 influencing the glutamate downstream signals, may be involved in pathophysiology of psychosis (Hahn et al. 2006).

Arc.

Activity-Regulated Cytoskeleton-associated Gene (*Arc*) is a member of the immediate-early gene family induced also in the presence of protein synthesis inhibitors (Link et al. 1995; Lyford et al. 1995). The *Arc* gene is located on chromosome 8 in the human and 7 in the rat, and it is highly conserved across vertebrate species. Arc proteins are localized

to activated synaptic sites in a NMDA-R dependent way (Steward and Worley 2001), has a spectrin homology sequence in C-terminal region, and binding sites for endophilin 3 and dynamin 2. Arc interacts with proteins involved in clathrin-mediated endocytosis and facilitates the removal of AMPA-R from the membrane (Chowdhury et al. 2006; Bloomer et al. 2007). Indeed, Arc is a marker for neuronal plastic changes relevant for learning and memory (McIntyre et al. 2005).

c-fos.

Although not a member of PSD, c-fos has great relevance for molecular neuroimaging studies, as it represents a well studied marker of neuronal activity during behavioral procedures or after drug administration. *c-fos* is a cellular proto-oncogene belonging to the IEG family of Transcription Factors. c-fos protein has a leucine-zipper DNA binding domain, and a C-terminal transactivation domain, by which it can dimerize with c-jun to form the AP1 transcription factor. *c-fos* gene is up-regulated in response to many signals involved in proliferation and differentiation. In neuroscience, *c-fos* expression is an indirect marker of neuronal activity because *c-fos* is often expressed when neurons fire action potentials. Indeed, *c-fos* mRNA is upregulated in neuron exhibiting recent activity (Nichols and Sanders-Bush 2002; Day et al. 2008; VanElzakker et al. 2008).

Glutamatergic mechanisms of antipsychotic drugs and new antipsychotic compounds with glutamatergic action.

Reports on glutamate involvement in psychosis raised the issue that drugs used in psychosis may also act on glutamatergic neurotransmission. A growing body of evidence is confirming that typical and atypical antipsychotics may exert, at least in part, an action on glutamatergic system. Typical and atypical antipsychotics modify extracellular level of glutamate, increase or decrease glutamate transporter and receptor gene expression and modify the glutamatergic receptors density as adaptive response of synapses (de Bartolomeis et al. 2005). However, antipsychotic agents acting primarily on glutamatergic neurotransmission are still lacking, most likely because of the risk of epileptogenesis and excitotoxicity with direct stimulation and/or blockade of glutamate receptors (Wasterlain and Chen 2008; Qian et al. 2011).

Therefore, a number of compounds modulating, rather than activating or blocking glutamate receptors, are currently on examination.

The NMDA-R is structurally complex and exhibits several modulation sites. Among them, the strychnine-insensitive glycine-binding site, by which the glutamate co-agonist glycine may positively modulate NMDA-R opening. Previous studies have explored the possibility to modulate NMDA-R function, facilitating glutamatergic neurotransmission by means of NMDA-Rs co-agonists (glycine, D-serine, D-cycloserine) or blocking the re-uptake of glycine on astrocyties through glycine transporter inhibitors (N-methyl glycine, called sarcosine).

Recently, a class of compounds acting as selective antagonists at NR2 subunits by an allosteric interaction with NR2 extracellular domain has been described (Malherbe et al. 2003). These compounds are predominantly selective for NR2B subunits and are derivatives of ifenprodil, the first selective NR2B allosteric antagonist described.

Ifenprodil-derivatives have been proposed in clinical trials in the therapy of traumatic brain injury. The polyamines spermine and spermidine act as positive allosteric modulators of NMDA-R by binding the so-called polyamine binding site. Polyamines facilitate NMDA-R function by a glycine-dependent (enhancement of receptor affinity for glycine) and a glycine-independent NR2B-dependent mechanism (Williams 1997). A positive allosteric modulation of NMDA-R has also been reported for the endogenous neurosteroid pregnenolone sulphate, which seems to act by NR2A and NR2b subunits (Malayev et al. 2002).

Another class of compounds that has received great attention has been that of AMPAkines. AMPAkines are AMPA-R agonists that increase the peak and the duration of open-channel phase playing a role in starting the events responsible for synaptic plasticity as long-term potentiation mediated by NMDA-R activation. AMPAkines potentiate synergistically the activity of typical and atypical antipsychotics in blocking methamphetamine-induced locomotor activity and behavioral modifications in rats (Johnson et al. 1999).

Another suggested strategy has been to modulate metabotropic glutamate receptors. mGluR5 is a postsynaptic receptor involved in neuron activation involved in depolarization, increases of NMDA-R currents, increases of firing frequency and burst-firing activity (Awad et al. 2000). mGluR5 amplification of NMDA-R-mediated responses provides the basis to rescue NMDA-R hypo-glutamatergic neurotransmission. Positive allosteric modulators of mGluR5, such as ADX47273, prevent PCP- and amphetamine-induced hyperlocomotion (Liu et al. 2008).

One of the most recent therapeutic strategies suggest to use glutamate release inhibitors (GRIs): antiepileptic drugs (above all lamotrigine and topiramate) (Dursun and Devarajan 2001; Tiihonen et al. 2003; Afshar et al. 2009); L-type calcium channel

antagonist (nimodipine); the mGluR2/3 agonist LY354740, that has been described to counteract ketamine-induced disruption of working memory in healthy humans (Krystal et al. 2005); inhibition of glutamate carboxipeptidase II (GCP-II). GCP-II is the enzyme that degrades the endogenous agonist of mGluR2/3, N-acetylaspartylglutamate (NAAG). Therefore inhibition of GCP-II may increase concentration of endogenous NAAG, thus representing a potential strategy for mGluR2/3 activation (Krystal et al. 2003).

Growing interests are captured also by the NMDA-R partial antagonist memantine (Krystal et al. 2003). Memantine- NMDA-R interaction allows physiological activation of NMDA-R and inhibits pathological over-activation of NMDA-R (Johnson and Kotermanski 2006). Indeed, memantine decreases in synaptic noise resulting from excessive NMDA-R activation, allows the physiological synaptic signaling and re-balances between inhibition and excitation of glutamate system (Parsons et al. 1999; Parsons et al. 2007; Gilling et al. 2009). Memantine is approved by the Food and Drug Administration for treatment of moderate to severe Alzheimer Disease and is used in clinical practice for treatment of other neuropsychiatric disorder associated with excitotoxic cell death such as Parkinson's disease, amyotrophic lateral sclerosis, epilepsy, stroke, spasticity, vascular dementia, in off-label use for psychosis. Memantine is also being tested for generalized anxiety disorder, depression, obsessive compulsive disorder, bipolar disorder, substance abuse, binge eating disorder, Attention Deficit Hyperactivity Disorder, pervasive developmental disorders (Zdanys and Tampi 2008), Tourette Syndrome, HIV associated dementia, multiple sclerosis, glaucoma, neuropathic pain syndrome, opioid dependence, systemic lupus erythematous. Memantine reduces or prevents excitotoxic damage without producing undesired side

effects, such as hallucination, agitation, catatonia, centrally mediated increase in blood pressure, anesthesia (Zdanys and Tampi 2008).

Aims of the research.

In the studies described herein, we explored in three different paradigms the expression of multiple genes relevant for dopamine and glutamate neurotransmission, glucose metabolism and brain region specific activation (i.e. activation of inducible early genes) in an animal model of psychosis, in which glutamatergic neurotransmission was perturbed by NMDA-R blockade or modulation following in vivo administration of sub-convulsing and sub-anesthetic doses of ketamine.

The overall strategy beyond this approach stemmed mainly from the observation that in vivo human studies have shown in psychotic patients or in normal subject after ketamine administration a disruption of dopamine-glutamate interaction, an abnormal glucose metabolism in cortical and subcortical areas and a dysfunctional activation of cortico-striatal regions (Kegeles et al. 2002; Erritzoe et al. 2003).

We chose NMDA-R blockade or modulation as putative animal model of psychosis not only on the basis of the results from human studies but also because previous studies have extensively shown that NMDA-R antagonism after administration of ketamine, is responsible of neuronal pathological changes in rat brain regions believed to be relevant in modeling the pathophysiology of schizophrenia. We chose NMDA-R blockade or modulation as putative animal model of psychosis not only on the basis of the results from human studies but also because previous studies have extensively shown that NMDA-R antagonism after administration of ketamine, is responsible of neuronal pathological changes in rat brain regions believed to be relevant in modeling the pathophysiology of schizophrenia (Prabakaran et al. 2004; McDermott and de Silva 2005; Robey and Hay 2006; Martins-de-Souza et al. 2009; Martins-De-Souza et al. 2010).

Considering the relevance of spatial analysis to gain insights in the molecular perturbation that putatively occurs after administration of ketamine, a molecular imaging approach at level of transcripts was using of radioactive quantitative in situ hybridization.

Therefore, the animal model provided by NMDA-R blockade may allow studying molecular dysfunctional putatively implicated in psychosis pathophysiology.

As NMDA-R blockade affects glutamatergic neurotransmission, it is expected that several other neurotransmitter systems, above all the dopaminergic may be affected by administration of this compound, thus raising the possibility to investigate the perturbation of the interplay between neurotransmitter systems. It has indeed suggested that psychosis, rather than depending on alteration in a single neurotransmitter system, may result from a complex imbalance between several systems, including the dopaminergic and the glutamatergic ones.

The set of studies carried out and reported here has been specifically designed to investigate these issue.

After having reported the experimental procedure (chapter 2). we will describe each specific experimental paradigm and corresponding results:

a) Modulation of Hk1 and GLUT 3 in an animal model of psychosis: a putative link between metabolism and behavioral disorders (chapter 3).
b) Modulation of Dopamine Receptors type 1-2 and Dopamine Transporter in an animal model of psychosis: acute and subchronic paradigms (chapter 4).
c) Modulation of constitutive and inducible early genes implicated in glutamate neurotransmission by different antagonists at NMDA receptors (chapter 5).

Chapter 2.

Experimental procedures.

Animals and treatments.

Male Sprague–Dawley rats (Charles-River Laboratories, Lecco, Italy) of approximately 250- 300g were housed in a temperature and humidity controlled colony room.

"Metabolic Genes": rats were randomly assigned to one of the following treatment groups: (1) saline solution 0.9% NaCl (VEH); (2) Ketamine 12 mg/kg (KET12); (3) Ketamine 50 mg/kg (KET50). There were four rats for each treatment group. Animals were sacrificed by decapitation 180 minutes after the treatment.

"Dopamine genes":

Acute paradigm: rats were randomly assigned to one of the following treatment groups: (1) saline solution 0.9% NaCl (VEH); (2) Ketamine 12 mg/kg (KET12); (3) Ketamine 50 mg/kg (KET50). There were four rats for each treatment group. Animals were sacrificed by decapitation 180 minutes after the treatment.

Subchronic paradigm: rats were randomly assigned to one of the following treatment groups: (1) saline solution 0.9% NaCl (VEH); (2) Ketamine 12 mg/kg (KET12) with 1 injection/day per 7 days. There were four rats for each treatment group. Animals were sacrificed by decapitation 180 minutes after the last injection.

"Postsynaptic density Genes": on the day of the experiment, rats were randomly assigned to one of the five following groups: (1) saline solution 0.9% NaCl (VEH); (2) memantine 5 mg/kg (MEM); (3) MK-801 0.8 mg/kg; (4) ketamine 25 mg/kg (KET25);

(5) ketamine 50 mg/kg (KET50). Each experimental group was composed of seven rats. The animals were sacrificed by decapitation 90 minutes after the treatment.

All drugs were dissolved in a vehicle of physiological saline solution (0.9% NaCl), which was used as a control. Drug doses were chosen based on previous animal studies in which molecular and behavioral effects predictive of either pro-psychotic or anti-psychotic activity were elicited (Iasevoli et al. 2007; Parsons et al. 2007). Ketamine was administered in sub-anaesthetic doses (a mild-to-moderate sub-anaesthetic, 12 and 25 mg/kg, and a high sub-anaesthetic and sub-convulsant dose, 50 mg/kg), known to provide an animal model of psychosis and to affect NMDA-R-mediated glutamate neurotransmission. Ketamine doses are in the range of those demonstrated to elicit behavioral effect as well as to induce early gene activation (Iasevoli et al. 2007). The dose of MK-801 is based on the behavioral and neurochemical effect both in acute and in chronic of this compound.

The dose of memantine (5 mg/kg i.p.) in the rats was the acute dose giving serum concentrations at 20 and 30 minutes corresponding to the upper limit of those observed in the serum of patients and healthy volunteers following treatment with well-tolerated doses of memantine (Parsons et al. 2007).

All solutions were suited to physiological pH value and injected intraperitoneally (i.p.) at a volume of 1 ml/kg (300μL). All procedures were conducted in accordance with the NIH Guide for Care and Use of Laboratory Animals (NIH Publication N0. 85-23, revised 1996) and were approved by local Animal Care and Use Committee. All efforts were made to minimize the number of animals and their sufferance.

Tissue sectioning.

After the killing, the brains were removed, frozen on dry ice and stored at −45 ◦C. Coronal sections of 12µm were cut on a cryostat -20°C, using the rat brain atlas by Paxinos and Watson (Paxinos and Watson, 1997) at the level of the middle-rostral striatum (approximately from Bregma -0.2 mm to -1.00 mm), of hippocampus (rostral regions of retrosplenial cortex; approximately from Bregma -3.00 mm to -4.50 mm), of substantia nigra/VTA (caudal regions of retrosplenial cortex- midbrain; approximately From -4.50 mm to -6.20 mm). Sections were thaw-mounted onto gelatine-coated slides; subsequently, they were processed for radioactive *in situ* hybridization.

Radiolabeling and purification of oligonucleotide probes.

The *Hk1* probe was a specifically designed 48-base oligodeoxyribonucleotide complementary to bases 827-874 of the rat *Hk1* mRNA.

The *GLUT3* probe was a 48-base oligodeoxyribonucleotide complementary to bases 1511-1558 of the rat *GLUT3* mRNA (GenBank Accession n. D13962).

The *D1R* probe was a 32-base oligodeoxyribonucleotide complementary to bases 829-861 of the rat *D1R* mRNA (GenBank Accession n. #434).

The *D2R* probe was a 48-base oligodeoxyribonucleotide complementary to bases 7-28 of the rat *D2R* mRNA (GenBank Accession n. #436).

The *DAT* probe was a 48-base oligodeoxyribonucleotide complementary to bases 871-918 of the rat *DAT* mRNA (GenBank Accession n. NM012694).

The *Homer1a* probe was a 48-base oligodeoxyribonucleotide complementary to bases 2527–2574 of the rat *Homer1a* mRNA (GenBank Accession n. U92079).

The *Homer1b* probe was a 48-base oligodeoxyribonucleotide complementary to bases 1306-1353 of the rat *Homer1b* mRNA (GenBank Accession n. AF093268).

The *PSD95* probe was a 45-base oligodeoxyribonucleotide complementary to bases 225-269 of the rat *PSD95* mRNA (GenBank Accession n. M96853).

The *c-fos* probe was a 48-base oligodeoxyribonucleotide complementary to bases 270-319 of the rat *c-fos* mRNA (GenBank Accession n. NM022197.2).

The *Arc* probe was a 45-base oligodeoxyribonucleotide complementary to bases 833-878 of the rat *Arc* mRNA (GenBank Accession n. NM019361).

The specificity of each probe was also tested by pilot control experiment using the corresponding sense oligodeoxyribonucleotide. All the oligodeoxyribonucleotides were purchased from MWG Biotech (Firenze, Italy). For each probe a 50 µl labeling reaction mix was prepared on ice using DEPC-treated water, 1X tailing buffer (metabolic paradigm) or 5X tailing buffer (PSD paradigm), 7.5pmol/µl (metabolic paradigm) or 5 pmol/µl (PSD paradigm) of oligodeoxyribonucleotide, 125 units (metabolic paradigm) or 2,5 units (PSD paradigm) of terminal deoxynucleotidyl transferase (TdT) and 100 mCi 35S-dATP. The mix was incubated 20 min at 37°C. The unincorporated nucleotides were separated from radiolabeled DNA using ProbeQuant G-50 Micro Columns (Amersham–GE Healthcare Biosciences; Milano, Italy).

In situ hybridization procedures.

Sections were processed for radioactive *in situ* hybridization (Ambesi-Impiombato et al. 2003; Tomasetti et al. 2007; Dell'aversano et al. 2009). All solutions were prepared with sterile double-distilled water. The sections were fixed in 4% formaldehyde in 0.12 M phosphate-buffered saline (1X PBS, pH 7.4) for five minutes, quickly rinsed twice with 1X PBS, and placed in 0.25% acetic anhydride in 0.1 M triethanolamine, 0.9% NaCl, pH 8.0, for 10 min at room temperature. Next, the sections were dehydrated in 70%, 80%, 95% and 100% ethanol, delipidated in chloroform for 5 min, rinsed again in 100%

and 95% ethanol and air dried. Sections were hybridized with 0.4–0.6x10^6 c.p.m. of radiolabeled oligonucleotide in buffer containing 50% formamide, 600mM NaCl, 80mM Tris–HCl (pH 7.5), 4mM EDTA, 0.1% pyrophosphate, 0.2% SDS, 0.2 mg/ml heparin sulfate, and 10% dextran sulphate, 100 mM DTT 5M. Slides were covered with coverslips and incubated at 37°C in a humid chamber for 22 h. After hybridization the coverslips were removed in 1X SSC (saline sodium citrate solution) and the sections were washed four times of 15 min in 2X SSC/50% formamide at 43–44 °C, followed by two 30 min washes with 1X SSC at room temperature. The slides were rapidly rinsed in distilled water and then in 70% ethanol.

Image capture and statistical analysis.

Hybridized sections were dried and exposed to Kodak-Biomax MR Autoradiographic film (Sigma-Aldrich). A slide containing a scale of 16 known amounts of 14C standards (American Radiolabeled Chemicals, St. Louis, MO, USA) was co-exposed with the samples. The autoradiographic films were exposed in a time range of 14–30 days. The optimal time of exposure was chosen to maximize signal-to-noise ratio but to prevent optical density from approaching the limits of saturation. Film development protocol included a 1.5 min dip in the developer solution, a 30 seconds rinse in water, a 3 min in the fixer and a 30 seconds rinse in water.

Image analysis.

The quantitation of the autoradiographic signal was performed using a computerized image analysis system including: a transparency film scanner (Microtek Europe B. V., Rotterdam, The Netherlands), an Apple PowerPC G4, and ImageJ software (v. 1.40, Rasband, W.S., http://rsb.info.nih.gov/ij/). Sections on film were captured individually.

The original characteristics of the scanned images (i.e. contrast, brightness, resolution) were preserved. All hybridized sections were exposed on the same sheet of X-ray film. Signal intensity analysis was carried out on digitized autoradiograms measuring mean optical density within outlined regions of interest (ROIs; Fig.1) in correspondence of subregions of the cortex (frontal, retrosplenial, enthorinal, visual, auditory cortices), caudate putamen, nucleus accumbens, ventro tegmental area, substantia nigra, midbrain. Sections were quantitated blind to the treatment conditions. In order to test for inter-observer reliability an independent quantitation was performed by a second investigator. Results obtained by the first investigator were considered reliable, and then reported, only when they were quantitatively comparable, in terms of consistency of the statistically significant effects found, to that obtained by the second investigator.

Data processing.

Measurements of mean optical density within ROIs were converted using a calibration curve based on the standard scale co-exposed to the sections. 14C standard values from 4 through 12 were previously cross-calibrated to 35S brain paste standards, in order to assign a d.p.m./mg tissue wet weight value to each optical density measurement through a calibration curve. For this purpose a "best fit" 3rd degree polynomial was used. For each animal, measurements from the three adjacent sections were averaged and the final data were reported in relative d.p.m. as mean +/- S.E.M. A One-Way Analysis of Variance (ANOVA) was used to analyze treatment effects. The Student's t test and the Student-Newman-Keuls post hoc test was used to determine the locus of effects in any significant ANOVA.

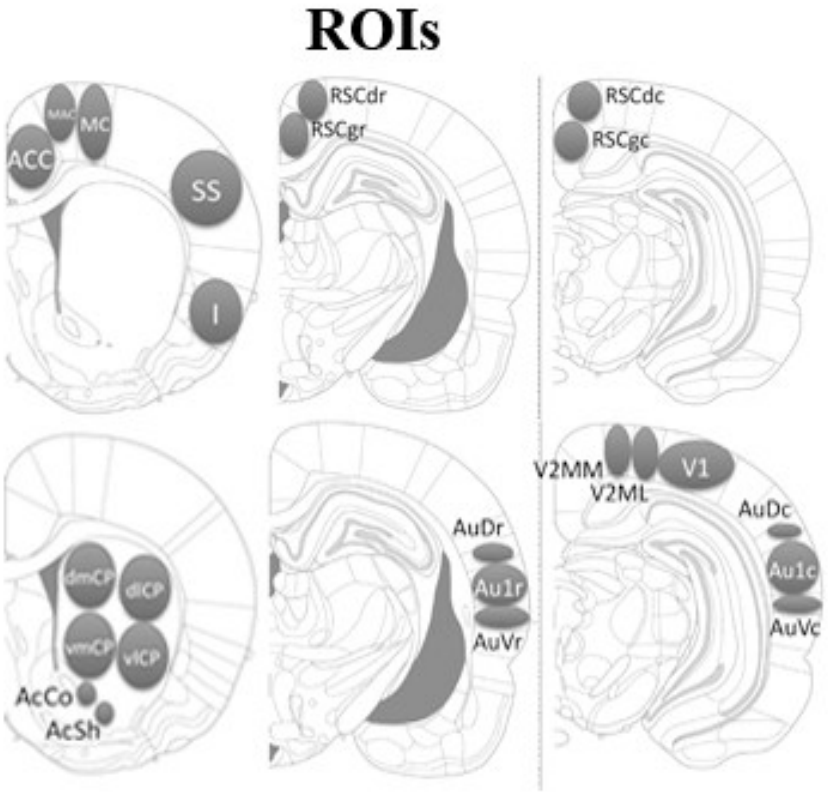


Figure 1. Region of Interests (ROIs)

Chapter 3.

Modulation of Hk1 and GLUT3 in an animal model of psychosis: a putative link between metabolism and behavioral disorders.

Rationale.

Schizophrenia has long been associated to an impairment of glucose regulation. Co-occurrence of schizophrenia and type 2 diabetes mellitus has been described (Bellivier 2005), which may be due to common genetic and environmental factors. Given their involvement in both glucose metabolism and cognitive functions, a cluster of putative genes for both schizophrenia and type 2 diabetes mellitus, including *glycogen synthase kinase 3* (*GSK-3*), *tyrosine hydroxylase* (*TH*) and *dopamine D2 receptor* (*D2R*), is currently under investigation (Lin and Shuldiner 2010).

Molecules involved in intracellular glucose metabolism may also be implicated in psychosis or in the mechanism of action and side effects of antipsychotics (Beaulieu et al. 2004; Beaulieu et al. 2005; Chen et al. 2007). Among key genes of glucose metabolism that have been associated with psychosis are Hexokinase1 and Glucose Transporter type 3.

Hexokinases (Hk, ATP:D-esose 6-phosphotransferase) catalyze the phosphorylation of glucose to glucose-6-phosphate (G6P), the first and limiting reaction in glucose metabolism necessary for intake of glucose through glucose transporters and for subsequent glycolysis and glycogen synthesis (Yano et al. 1991; Roncero et al. 2000; Wilson 2003).

Hk 1,2,3 isoenzymes are about 100 KDa, have high affinity for glucose and are regulated by feedback by G6P; Hk4 isoenzyme is about 52KDa, exhibits lower glucose affinity and is not regulated by feedback (Roncero et al. 2000). Hk isoforms have

substantial conservation of amino acid sequences and may be differentiated according to biochemical and pharmacological features, including tissue distribution: Hk1 shows ubiquitous tissue distribution. High levels of *Hk1* mRNA expression and immunoreactivity have been demonstrated within brain tissues (Jacobsson and Meister 1994; Coerver et al. 1998)). Hk2 is distributed in insulin-sensible tissues, such as muscle and adipocyte; Hk4 is distributed in hepatocytes and in pancreatic islands (Cardenas et al. 1998).

Hk1 and Hk2 are saturated by normal blood glucose concentration and are even activated when glucose concentration is lower than the physiologic range, in order to preserve the brain metabolism.

Hk3 activity is inhibited by high levels of blood glucose. Hk4 activity depends on variation of glucose concentration within the blood range (Cardenas et al. 1998).

Within the subcellular fraction, Hk1 is localized at the outer portion of mitochondria membrane (Polakis and Wilson 1985; Xie and Wilson 1988; Sui and Wilson 1997; Wilson 2003; Golestani et al. 2007) and acts in neurons also as an anti-apoptotic agent via its interaction with the voltage-dependent anion channel-1 (VDAC-1) protein (Pastorino and Hoek 2003; Wilson 2003; Pastorino and Hoek 2008). Intriguingly, the Hk1 interaction with mitochondria, which is required to prevent apoptosis, is mediated by the serine-threonine kinase Akt (Martins-De-Souza et al. 2010) and by GSK3b (glycogen- synthase- kinase) that have been implicated in pathophysiology of psychosis and in response to antipsychotics (Emamian et al. 2004; Lang et al. 2007; Pastorino and Hoek 2008). The *Hk* gene has been found in linkage with schizophrenia susceptibility regions of the genome (Stone et al. 2004).

The glucose transporters' family is composed by co-transporter Sodium-Glucose and Glucose Transporter (GLUT). GLUTs are involved in a passive mechanism of

facilitated glucose diffusion. Among all GLUT isoforms, GLUT1 (high molecular weight isoform) is expressed by capillary of blood-brain barrier, GLUT2 (low molecular weight isoform) in glial cells, and GLUT3 in neurons (Leino et al. 1997; Vannucci et al. 1997; McEwen and Reagan 2004; Simpson et al. 2007).

GLUT3 is the most abundant in the brain, while its expression is limited in other tissues such as sperm, embryo, leucocyte, tumor cells (Maher et al. 1991; McCall et al. 1994; Leino et al. 1997; Vannucci et al. 1997). GLUT3 expression increases during synaptogenesis and during neuronal activity. GLUT3 has high affinity for glucose (Palfreyman et al. 1992; Nishimura et al. 1993; Maher et al. 1996). A deficit in membrane-bound glucose transporter proteins, as GLUT1 and GLUT3, has recently been proposed as a putative mechanism in Alzheimer disease and in schizophrenia (Simpson et al. 1994; Miyamoto et al. 2001; Simpson et al. 2008). Moreover, antipsychotics have been reported to impair glucose uptake and increase *GLUT3* expression, after long term exposure, in cultured cells (Dwyer et al. 1999).

This body of evidence let suppose that Hk1 and GLUT3 may be implicated in neuro-psychiatric diseases, as psychosis. In this study, we aimed to evaluate the expression of *Hk1* and *GLUT3* in rat brain areas relevant for schizophrenia pathophysiology and therapy after exposure to ketamine, a compound known to induce a state of NMDA-R hypofunction which may represent an animal model of psychosis.

Results.

Expression of *Hk1* and *GLUT3* genes has been investigated in rat brain regions of interest considered relevant for ketamine-induced psychosis in humans and/or for ketamine-mediated neurotoxicity in rats, that is believed to model psychosis mechanisms (Table 1-2; Fig.2-3-4-5).

Frontal cortex.

Hk1 mRNA expression was not significant different among groups, with the exception of the somatosensory cortex where KET50 significantly increased *Hk1* mRNA expression compared to VEH.

GLUT3 gene expression was significantly increased by KET12 compared to both KET50 and VEH in the somatosensory and insular cortices and compared to KET50 only in the anterior cingulate, medial agranular, and motor cortices.

Retrosplenial cortex.

Hk1 expression was significantly increased by KET50 compared to VEH and KET12 in granular and dysgranular caudal cortices and by KET50 compared to VEH in granular rostral cortex, while no significant differences were detected in agranular rostral cortex.

GLUT3 gene expression was not significantly affected by ketamine.

Enthorinal cortex.

Both *Hk1* and *GLUT3* gene expression was not significantly affected by ketamine in this region.

Auditory cortex.

Quantization carried out in the caudal sections showed that *Hk1* gene expression was significantly increased by KET50 compared to both KET12 and VEH in the primary auditory cortex and in the ventral area of the secondary auditory cortex, while no significant differences among groups were observed in the dorsal area of the secondary auditory cortex. In the ventral area of the secondary auditory cortex, KET12 significantly reduced *Hk1* expression when compared to vehicle.

No significant differences among groups as related to *GLUT3* expression were found in any of the auditory cortex subregion.

In the rostral sections, *Hk1* expression was significantly increased by both KET50 and KET12 compared to vehicle in all auditory cortex subregions. No significant differences between KET50 and KET12 were recognized.

GLUT3 expression was found unaffected by treatments in rostral sections of auditory cortex.

Visual cortex.

In primary and secondary (either mediomedial, mediolateral, or lateral) visual cortices, *Hk1* expression was found to be significantly increased by KET50 compared to both KET12 and vehicle.

GLUT3 expression was found to be not affected by treatments in these regions.

Striatum.

Expression of *Hk1* gene was significantly increased by KET50 compared to both KET12 and VEH in the dorsomedial, dorsolateral, and ventromedial caudate putamen

and in the core of nucleus accumbens. No significant differences were recognized in the ventrolateral caudate putamen and in the shell of the nucleus accumbens.

GLUT3 expression was significantly decreased by KET50 compared to both KET12 and VEH in dorsomedial, dorsolateral, and ventromedial caudate putamen; and compared to KET12 only in ventrolateral caudate putamen and in the shell of the nucleus accumbens. In the core of the nucleus accumbens, KET50 also reduced *GLUT3* expression compared to KET12, albeit only a trend toward significance (ANOVA, $p=0.057$) was found.

Lateral Septum.

Hk1 expression was not significantly affected by treatments in any of the lateral septum subregions.

GLUT3 expression was significantly decreased by KET50 compared to both KET12 and vehicle in the dorsal part of the lateral septum and compared to the KET12 only in the intermediate part of the lateral septum. No significant differences were recognized in the ventral part.

Substantia Nigra and Ventrotegmental area.

Hk1 expression resulted to be not significantly affected by treatments in both the ventrotegmental area and the substantia nigra (either pars reticulata or pars compacta), although a trend toward significance was observed in the substantia nigra pars reticulata (i.e.: *Hk1* expression increased by KET50 compared to KET12 and vehicle).

No significant differences among groups in *GLUT3* expression were found in these regions.

	KET50	KET12	VEH	ANOVA (df, p)
Frontal cortex				
Anterior Cingulate cortex	93.57±1.04	91.09±1.49	89.21±1.72	$F_{2,8}$=2.33 P=0.159
Medial Agranular Cortex	91.34±1.77	87.59±2.42	86.57±2.38	$F_{2,8}$=1.31 P=0.326
Motor Cortex	93.35±1.09	89.72±1.58	89.24±1.71	$F_{2,8}$=2.43 P=0.149
Somatosensory Cortex	90.43±0.96*	88.96±1.26*	84.59±1.37	$F_{2,8}$=5.88 **P=0.027**
Insular Cortex	94.78±1.11	94.01±1.51	85.62±7.01	$F_{2,8}$=2.01 P=0.197
Retrosplenial Cortex				
Rostral Granular Cortex	151.46±8.64*	126.81±3.55	117.162±7.17	$F_{2,7}$=6.35 **P=0.026**
Rostral Dysgranular Cortex	151.82±9.46	142.04±5.13	122.14±8.39	$F_{2,7}$=3.68 P=0.08
Caudal Granular Cortex	144.45±7.71**	109.13±2.83	122.39±6.56	$F_{2,8}$=8.86 **P=0.009**
Caudal Dysgranular Cortex	160.01±6.72**	119.81±5.88	129.23±7.67	$F_{2,8}$=8.41 **P=0.01**
Enthorinal Cortex	89.67±3.25	83.45±1.14	85.37±1.49	$F_{2,7}$=2.53 P=0.15
Rostral Auditory Cortex				
Primary Auditory Cortex	92.66±1.09*	92.95±1.25*	86.46±2.23	$F_{2,8}$=5.59 **P=0.0303**
Dorsal Secondary Auditory Cortex	92.09±1.41*	91.49±1.42*	83.86±1.27	$F_{2,8}$=9.49 **P=0.007**
Ventral Secondary Auditory Cortex	94.22±1.08*	93.15±1.73*	87.88±0.89	$F_{2,8}$=5.53 **P=0.031**
Caudal Auditory Cortex				
Primary Auditory Cortex	93.81±0.14**	88.51±1.37	89.27±1.13	$F_{2,7}$=6.11 **P=0.029**
Dorsal Secondary Auditory Cortex	91.83±0.66	87.63±2.88	85.86±2.18	$F_{2,7}$=1.53 P=0.28
Ventral Secondary Auditory Cortex	93.98±0.26**	87.76±0.88$^{\#}$	91.25±0.45	$F_{2,7}$=21.69 **P=0.001**
Visual Cortex				
Primary Visual Cortex	91.65±0.44**	86.37±1.13	84.65±1.36	$F_{2,7}$=10.33 **P=0.008**
Mediomedial Secondary Visual Cortex	91.71±0.35**	86.87±1.33	86.37±0.51	$F_{2,7}$=7.99 **P=0.015**
Mediolateral Secondary Visual Cortex	91.91±0.51**	86.87±0.48	85.63±0.73	$F_{2,7}$=31.16 **P=0.0003**

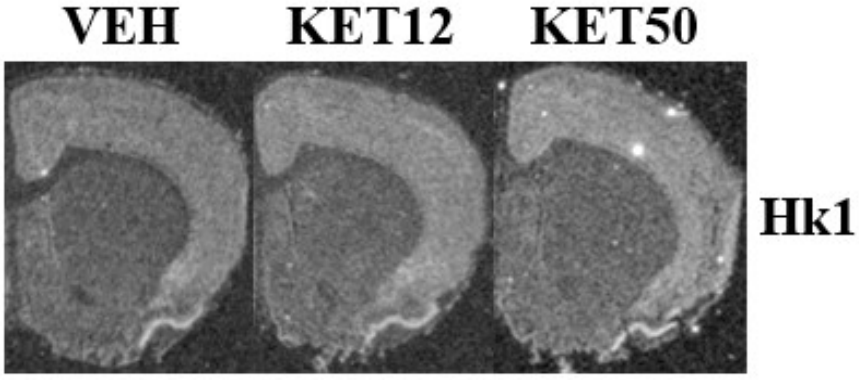
Lateral Secondary Visual Cortex	91.32±0.42**	87.26±0.84	84.44±1.81	$F_{2,7}$=8.65 **P=0.013**
Striatum				
Dorsomedial Caudate Putamen	74.89±1.34**	68.08±1.15	69.54±1.19	$F_{2,8}$= 8.74 **P=0.0097**
Dorsolateral Caudate Putamen	73.97±1.23**	68.37±1.85	66.79±0.78	$F_{2,8}$=6.63 **P=0.02**
Ventromedial Caudate Putamen	75.03±0.91**	70.29±1.88	68.87±1.16	$F_{2,8}$=5.03 **P=0.038**
Vetrolateral Caudate Putamen	74.28±1.39	70.23±2.58	66.49±1.14	$F_{2,8}$=3.79 **P=0.06**
Core of the Nucleus Accumbens	80.75±0.65**	72.18±1.96	73.28±0.47	$F_{2,8}$=12.87 **P=0.003**
Shell of the Nucleus Accumbens	81.18±0.98	75.49±3.31	75.81±0.71	$F_{2,8}$=2.16 P=0.18
Lateral Septum				
Dorsal Lateral Septal Nucleus	81.58±1.37	79.15±2.41	75.13±1.31	$F_{2,7}$=2.47 P=0.15
Intermediate Lateral Septal Nucleus	83.28±1.05	78.53±2.47	76.19±2.59	$F_{2,7}$=2.29 P=0.17
Ventral Lateral Septal Nucleus	84.75±1.97	79.98±2.29	77.61±3.76	$F_{2,7}$=1.63 p=0.26
Substantia Nigra/Ventrotegmental Area				
Substantia Nigra Pars Compacta	85.15±3.24	79.64±0.71	80.01±2.32	$F_{2,7}$=2.08 P=0.19
Substantia Nigra Pars Reticulata	64.87±0.74**	52.49±4.12	49.79±4.28	$F_{2,7}$=4.39 **P=0.05**
Ventrotegmental Area	88.64±1.59	84.99±2.96	81.07±0.26	$F_{2,7}$=2.49 P=0.15

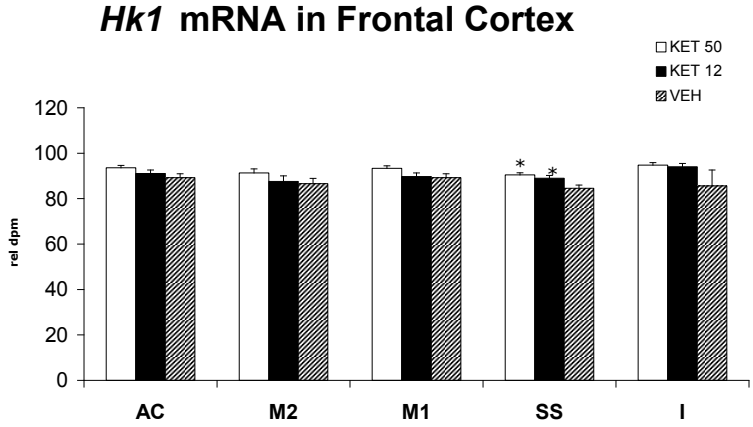
Table 1. mRNA levels of *Hexokinase I* after acute ketamine treatment. Data are expressed as d.p.m. mean values±standard error means (S.E.M.) and listed by brain regions analyzed, along with the relative ANOVA (degrees of freedom and p values). Significant p values are expressed in bold. Post-hoc test: ** significantly higher expression compared to KET12 and VEH; * significantly higher expression compared to VEH; # significantly lower expression compared to VEH.

	KET50	KET12	VEH	ANOVA (df, p)
Frontal cortex				
Anterior Cingulate cortex	72.99±1.08	83.38±2.61$^{\Diamond}$	79.88±2.19	$F_{2,8}$=5.14 **P=0.036**
Medial Agranular Cortex	68.81±3.18	81.17±0.96$^{\Diamond}$	75.39±2.03	$F_{2,8}$=8.66 **P=0.01**
Motor Cortex	66.76±4.24	80.59±1.98$^{\Diamond}$	76.11±2.76	$F_{2,8}$=5.41 **P=0.032**
Somatosensory Cortex	62.03±3.98$^{\#}$	80.68±2.04**	72.55±2.04	$F_{2,8}$=12.25 **P=0.003**
Insular Cortex	72.46±6.46	90.27±2.99**	76.34±1.79	$F_{2,8}$=6.49 **P=0.021**
Retrosplenial Cortex				
Rostral Granular Cortex	61.96±5.95	80.23±10.11	57.57±5.45	$F_{2,8}$=2.58 P=0.13
Rostral Dysgranular Cortex	61.88±4.79	82.04±11.85	59.08±5.02	$F_{2,8}$=2.53 P=0.13
Caudal Granular Cortex	61.83±3.42	67.90±8.54	62.34±9.70	$F_{2,7}$=0.16 P=0.85
Caudal Dysgranular Cortex	63.80±2.25	67.59±7.15	62.06±9.27	$F_{2,7}$=0.15 P=0.86
Enthorinal Cortex	70.62±6.14	84.44±0.88	73.86±5.71	$F_{2,7}$=2.21 P=0.19
Rostral Auditory Cortex				
Primary Auditory Cortex	73.65±0.78	82.01±1.35	66.61±6.81	$F_{2,7}$=3.64 P=0.09
Dorsal Secondary Auditory Cortex	72.09±1.07	80.91±2.34	64.13±6.79	$F_{2,7}$=3.99 P=0.07
Ventral Secondary Auditory Cortex	75.73±0.73	84.08±0.32	71.91±5.98	$F_{2,7}$=3.19 P=0.11
Caudal Auditory Cortex				
Primary Auditory Cortex	80.93±2.97	86.71±2.08	75.14±7.22	$F_{2,7}$=1.53 P=0.28
Dorsal Secondary Auditory Cortex	76.15±3.01	82.69±1.82	69.35±5.91	$F_{2,7}$=2.82 P=0.13
Ventral Secondary Auditory Cortex	81.54±2.85	88.63±3.35	75.35±8.21	$F_{2,7}$=1.53 P=0.29
Visual Cortex				
Primary Visual Cortex	75.72±1.29	83.78±2.63	68.76±8.43	$F_{2,7}$=2.13 P=0.2
Mediomedial Secondary Visual Cortex	74.65±3.22	84.46±2.24	71.34±7.91	$F_{2,7}$=1.79 P=0.24
Mediolateral Secondary Visual Cortex	73.37±2.12	85.15±2.37	70.58±7.09	$F_{2,7}$=2.97 P=0.12

Lateral Secondary Visual Cortex	74.13±2.67	82.98±1.45	70.22±8.58	$F_{2,7}$=1.55 P=0.28
Striatum				
Dorsomedial Caudate Putamen	50.24±1.35$^{\#}$	68.52±3.61$^{\Diamond}$	62.37±1.75	$F_{2,8}$= 11.34 **P=0.0046**
Dorsolateral Caudate Putamen	47.97±0.93$^{\#}$	63.57±2.41$^{\Diamond}$	58.21±1.29	$F_{2,8}$=17.77 **P=0.0011**
Ventromedial Caudate Putamen	46.56±1.83$^{\#}$	65.61±3.68$^{\Diamond}$	59.99±2.13	$F_{2,8}$=10.77 **P=0.0054**
Vetrolateral Caudate Putamen	45.11±0.71	64.98±4.25$^{\Diamond}$	54.95±3.06	$F_{2,8}$=8.21 **P=0.011**
Core of the Nucleus Accumbens	50.02±1.34	64.36±4.16$^{\Diamond}$	60.02±3.26	$F_{2,8}$=4.17 **P=0.05**
Shell of the Nucleus Accumbens	52.96±3.33	71.63±3.68$^{\Diamond}$	63.32±3.34	$F_{2,8}$=6.58 **P=0.021**
Lateral Septum				
Dorsal Lateral Septal Nucleus	48.39±1.31$^{\#}$	64.59±4.38$^{\Diamond}$	58.77±1.69	$F_{2,7}$=10.87 **P=0.007**
Intermediate Lateral Septal Nucleus	46.78±3.76	63.41±4.41$^{\Diamond}$	58.61±3.45	$F_{2,7}$=5.09 **P=0.04**
Ventral Lateral Septal Nucleus	55.97±2.61	67.53±4.76	63.45±2.88	$F_{2,7}$=3.17 p=0.11
Substantia Nigra/Ventrotegmental Area				
Substantia Nigra Pars Compacta	71.51±2.88	76.01±9.69	63.89±5.69	$F_{2,7}$=0.83 P=0.48
Substantia Nigra Pars Reticulata	42.02±1.92	53.58±9.95	42.95±9.08	$F_{2,7}$=0.66 P=0.54
Ventrotegmental Area	68.44±1.21	75.19±6.88	55.98±6.83	$F_{2,7}$=2.99 P=0.12

Table 2. mRNA levels of *Glucose Transporter 3* after acute ketamine treatment. Data are expressed as d.p.m. mean values±standard error means (S.E.M.) and listed by brain regions analyzed, along with the relative ANOVA (degrees of freedom and p values). Significant p values are expressed in bold. Post-hoc test: ** significantly higher expression compared to KET50 and VEH; $^{\Diamond}$ significantly higher expression compared to KET50; # significantly lower expression compared to VEH.





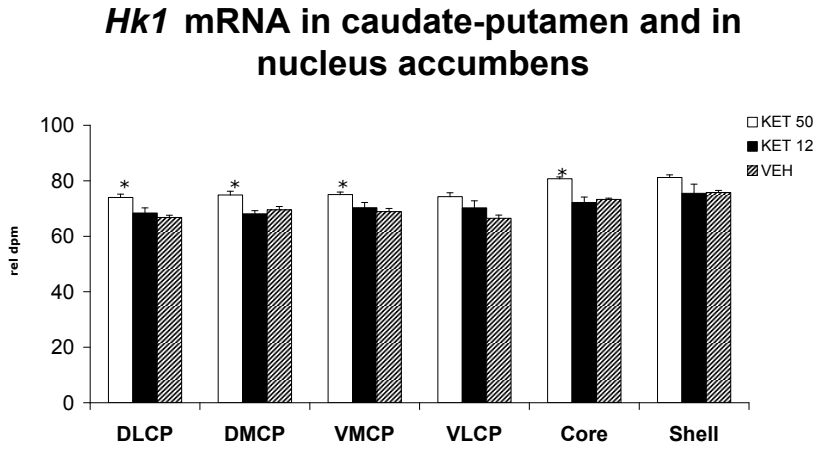
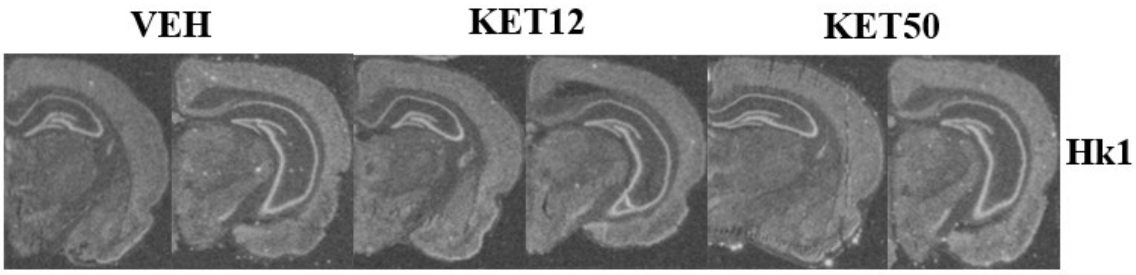
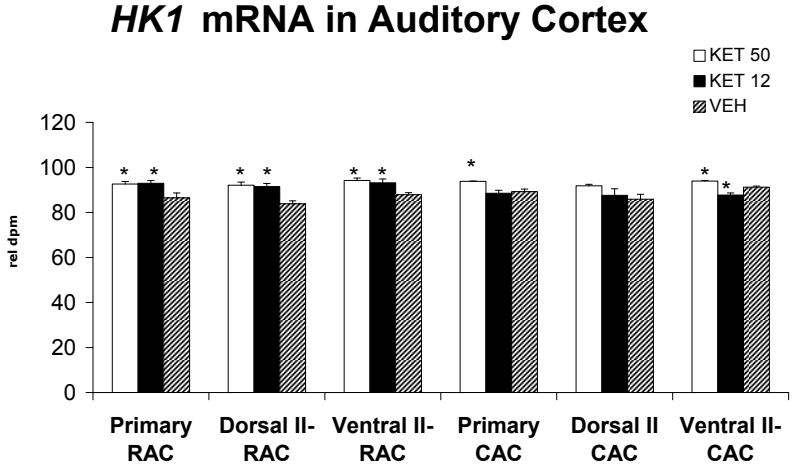
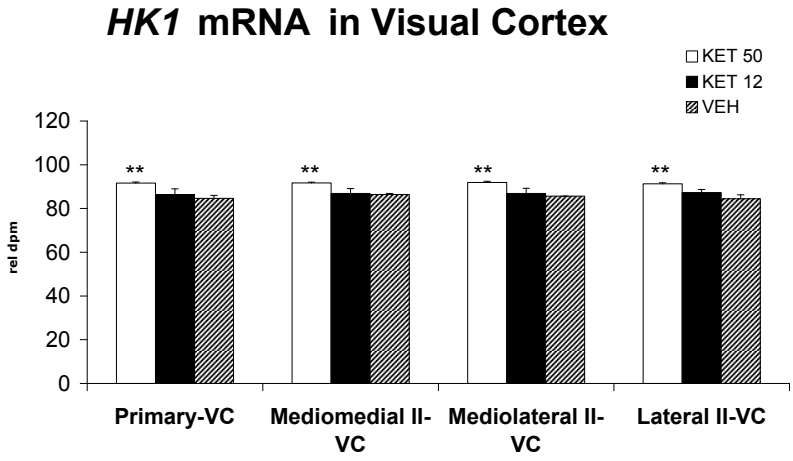


Figure 2. Autoradiographic images and graphics of *Hk1* mRNA expression in relative dpm as mean ± S.E.M.; *ANOVA $p<0,05$ vs VEH.


VEH
KET12
KET50
Hk1


HK1 mRNA in Auditory Cortex
KET 50
KET 12
VEH
rel dpm
120
100
80
60
40
20
0
Primary RAC
Dorsal II-RAC
Ventral II-RAC
Primary CAC
Dorsal II CAC
Ventral II-CAC


HK1 mRNA in Visual Cortex
KET 50
KET 12
VEH
rel dpm
120
100
80
60
40
20
0
Primary-VC
Mediomedial II-VC
Mediolateral II-VC
Lateral II-VC

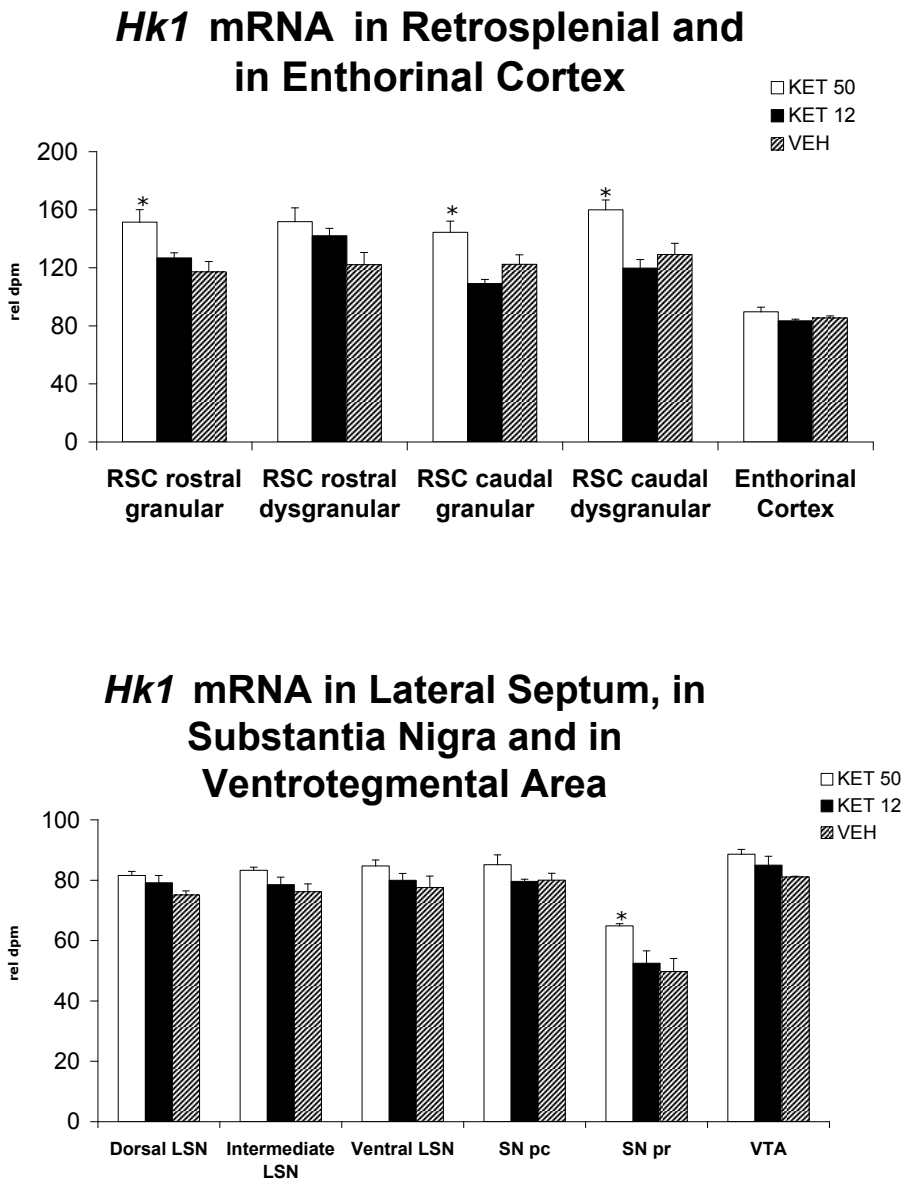
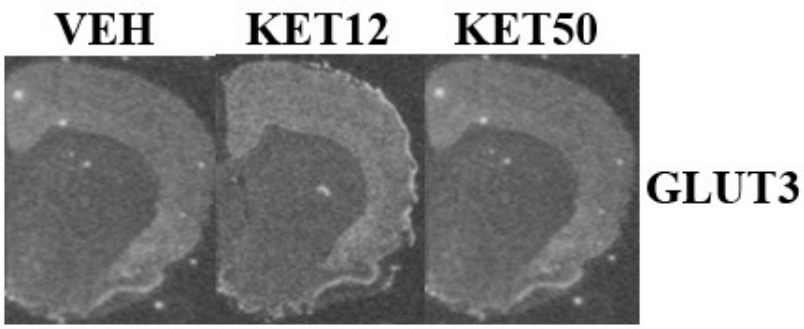
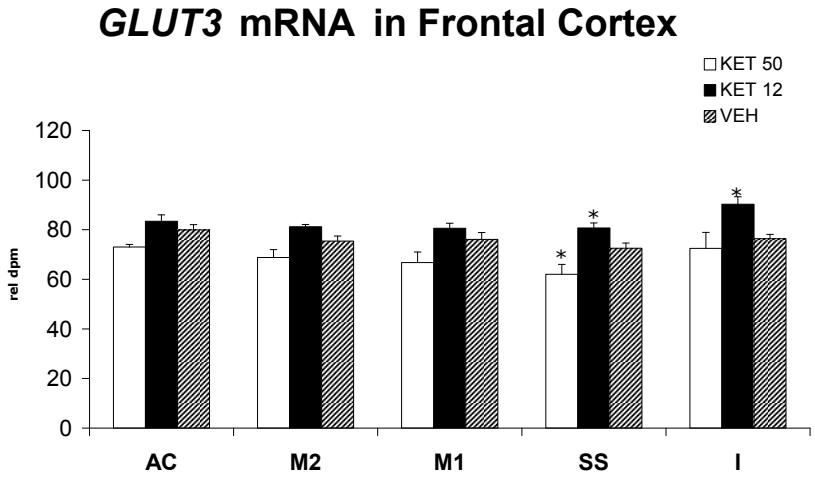


Figure 3. Autoradiographic images and graphics of *Hk1* mRNA expression in relative dpm as mean ± S.E.M.; *ANOVA $p<0,05$ vs VEH.





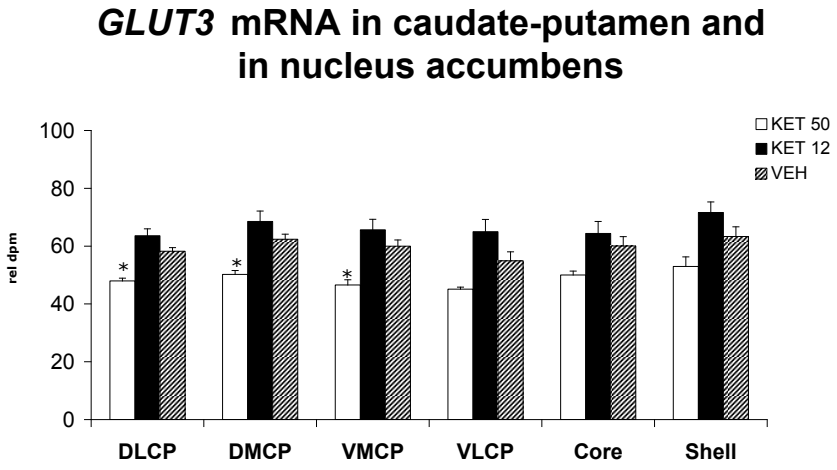
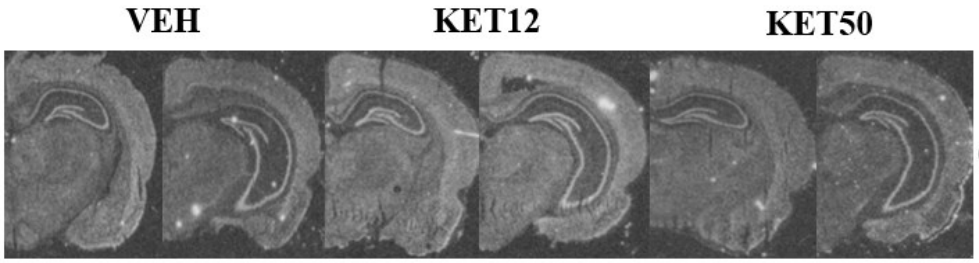
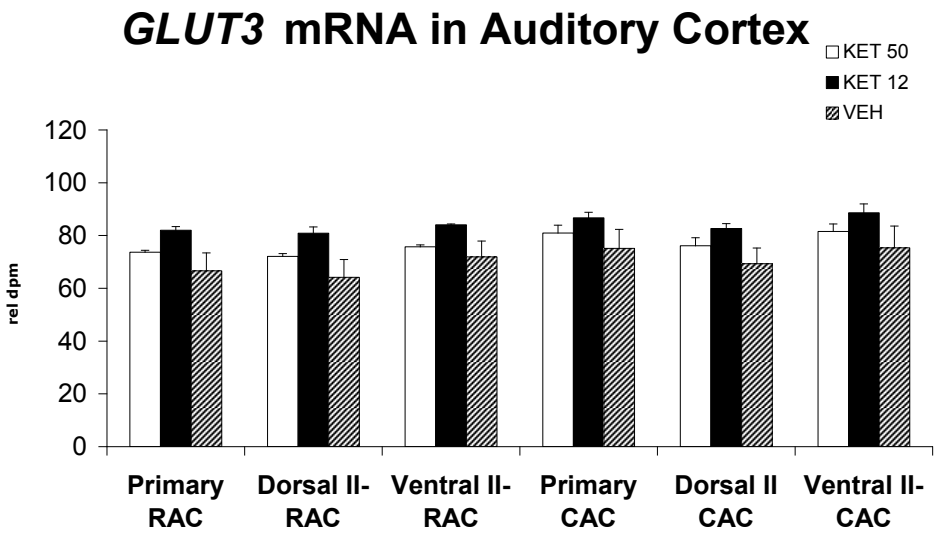
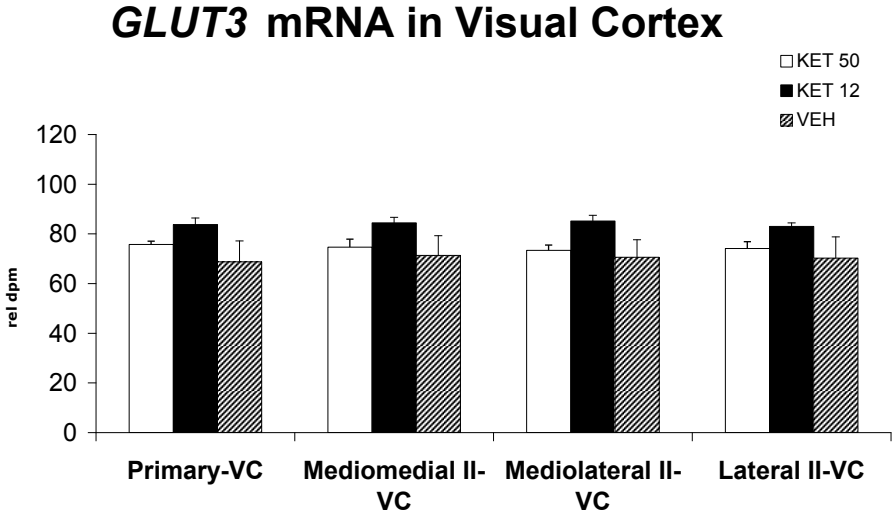


Figure 4. Autoradiographic images and graphics of *GLUT3* mRNA expression in relative dpm as mean ± S.E.M.; *ANOVA p<0,05 vs VEH.


VEH
KET12
KET50
GLUT3


GLUT3 mRNA in Auditory Cortex
KET 50
KET 12
VEH
rel dpm
120
100
80
60
40
20
0
Primary RAC
Dorsal II-RAC
Ventral II-RAC
Primary CAC
Dorsal II CAC
Ventral II-CAC


GLUT3 mRNA in Visual Cortex
KET 50
KET 12
VEH
rel dpm
120
100
80
60
40
20
0
Primary-VC
Mediomedial II-VC
Mediolateral II-VC
Lateral II-VC

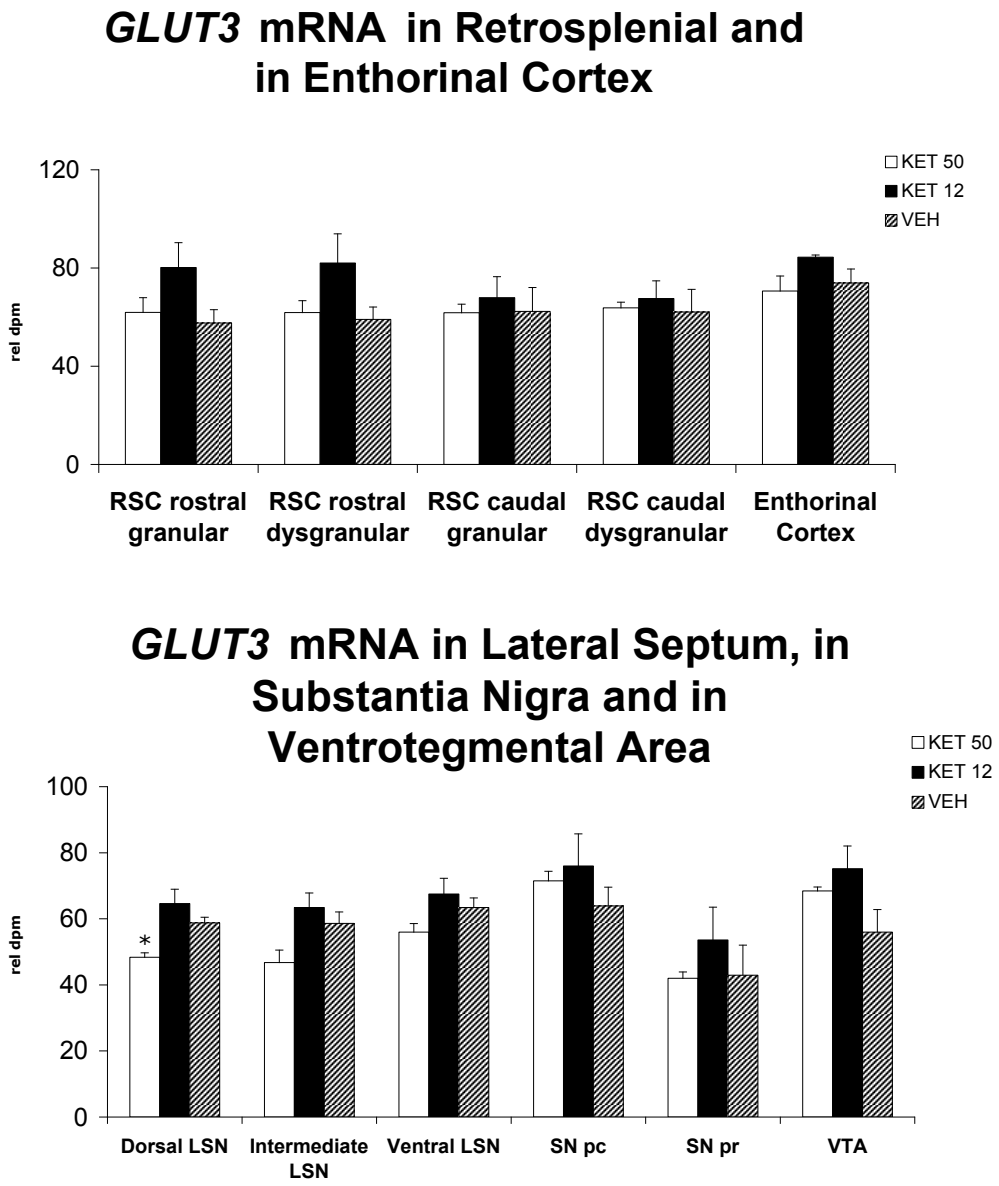


Figure 5. Autoradiographic images and graphics of *GLUT3* mRNA expression in relative dpm as mean ± S.E.M.; *ANOVA $p<0,05$ vs VEH.

Chapter 4.

Modulation of Dopamine Receptors type 1-2 and Dopamine Transporter in an animal model of psychosis: acute and subchronic paradigms.

Rationale

The imbalance between dopaminergic and glutamatergic neurotransmission at both cortical and subcortical level is regarded as one of the main pathophysiological mechanisms of psychosis (de Bartolomeis et al. 2005).

Moving from early suggestions that only implicated dopamine dysfunctions in psychosis, in recent years, e the glutamatergic hypothesis of psychosis pathophysiology has received even greater attention (Allen and Young 1978; Lodge and Anis 1982; Javitt and Zukin 1991). According to this hypothesis, a failure in glutamatergic neurotransmission, namely a NMDA-R hypofunction, may cause a complex cortical-subcortical perturbation involving several neurotransmitter systems, including the dopaminergic (Olney et al. 1999; Farber 2003). Consistent with glutamatergic hypothesis of psychosis, animal models provided by acute or subchronic administration of NMDA-Rs non-competitive antagonists, as ketamine, are considered to have strong predictive and construct validity (Lipska and Weinberger 2000). Thereby, these pharmacological models represent a powerful tool to study novel therapeutic targets and molecular basis of psychotic disorders and have allowed to carry out investigations on the dopamine-glutamate interplay in psychosis-mimicking paradigms.

Blockade of NMDA-R, by systemic NMDA-R blockers administration, has been reported to increase dopamine release in the cortex and ventral striatum in rats (Ikeda et al. 2011) and at least in striatum in humans (Kegeles et al. 2002). Chronic recreational users of ketamine have been demonstrated to suffer from a reduction of dopamine D1R

amount in the cortex (Narendran et al. 2005). Exposure to phencyclidine (PCP), a potent NMDA-R non-competitive antagonist, in rat puppets has been shown to decrease dopamine D2R binding in the striatum along different time points, as well as to slightly increase dopamine transporter (DAT) binding and tyrosine hydroxylase mRNA expression, suggesting a long-term increase of dopamine output. Moreover, it has been observed that antipsychotics, which are known to mainly act on dopamine neurotransmission, reverse the disruption in rat prefrontal cortex functions triggered by blockade of NMDA-Rs (Kargieman et al. 2007). It thus appears that dysfunctions in glutamate transmission may be associated to the perturbation of dopamine signaling and dopamine-mediated behaviors, which may be of relevance for psychosis pathophysiology. However, molecular mechanisms of glutamate-dopamine interplay in animal models of psychosis are yet to be exhaustively described. Moreover, some controversy is still under debate on whether psychosis may be best resembled after acute or subchronic administration of NMDA-R non-competitive antagonists. It is believed that acute administration of these compounds may induce neurochemical and behavioral manifestations consistent with acute positive and negative symptoms of psychosis (Gunduz-Bruce 2009). On the other hand, chronic exposure to NMDA-R non-competitive antagonists has been described to induce cortical dopaminergic and cognitive deficits in rats and monkeys, providing a model of cortical dysfunctions in psychosis (Jentsch et al. 1998). Moreover, subchronic PCP has been reported to induce hyperreactivity of striatal dopamine release and dopamine sensitization to psychostimulants (Jentsch et al. 1998; Balla et al. 2001), which may model the putative sustained perturbation of dopamine transmission occurring in schizophrenic individuals. In this study, our first goal was to verify whether perturbation of NMDA-R-mediated transmission may reflect in the perturbation of dopamine neurotransmission. To do so,

we investigated changes in gene expression in key molecules regulating dopamine signaling, namely the gene coding for dopamine D1 and D2Rs and for the DAT. As a second goal, we wanted to evaluate whether putative changes may be specific of one paradigm of ketamine administration (acute or subchronic, respectively) and whether putative changes were preserved or vary with the period of ketamine administration.

Dopamine receptors are a family of metabotropic G-protein coupled receptors. There are at least five subtypes of dopamine receptors, D1, D2, D3, D4, and D5. The D1 and D5 receptors are members of the D1-like family of dopamine receptors; activation of D1-like family receptors is coupled to the G protein $G_{\alpha s}$, which subsequently activates adenylyl cyclase, increasing the intracellular concentration of the second messenger cyclic adenosine monophosphate (cAMP), and activates cyclic AMP-dependent protein kinases. The D2, D3 and D4 receptors are members of the D2-like family; activation of D_2-like family receptors is coupled to the G protein $G_{\alpha i}$, which directly inhibits the adenylate cyclase decreasing the intracellular concentration of cAMP (Girault and Greengard 2004).

D1R is the most expressed dopamine receptors in the neocortex. D1R is a postsynaptic receptor of neurons involved in the meso-cortical pathway from the ventral tegmental area to the neocortex (Lewis and Howie 1987). Altered D1R function in dorso-lateral prefrontal cortex has been involved in the working memory impairment in schizophrenia (Weinberger 1987; Davis et al. 1991; Goldman-Rakic et al. 2000; Abi-Dargham et al. 2002).

D2R is encoded in two forms: the D2L form is a post-synaptic receptor, with both excitatory or inhibitory transmission functions; the D2S form is a presynaptic autoreceptor with modulatory functions by feed-back mechanisms, affecting synthesis, storage, and release of dopamine into the synaptic cleft. Low doses of an agonist prefer

the presynaptic and high doses prefer the postsynaptic D2Rs. When the dopamine in the synapse binds the presynaptic D2R, the presynaptic neuron is inhibited. Virtually all antipsychotic agents are antagonists at the D2R, except for aripiprazole that works as D2R partial agonist. D2R antagonism is functional to control positive symptoms of schizophrenia.

DAT is a pre-synaptic transporter involved in the clearance of dopamine in striatum regions. The rate at which DAT removes dopamine from the synapse can have effects on the amount of dopamine in the neuron. Dysfunction of DAT activity is involved in severe cognitive deficits and motor abnormalities.

In this study, we aimed to evaluate the expression of D1R, D2R and DAT in rat brain areas relevant for schizophrenia pathophysiology and therapy after perturbation of glutamate system in an acute and a subchronic paradigms.

Results.

D1R- Acute paradigm

Results, with respective ANOVA values and Student-Newman-Keuls post hoc test, graphics and autoradiographic images are shown in Fig.6.

A significant reduction of *D1R* gene expression was observed in the dorsolateral caudate putamen (**DLCP**, ANOVA, p=0.0473; F(2,8)=4.5791) and in the ventrolateral caudate putamen (**VLCP**, ANOVA, p=0.0253; F(2,8)=6.0282), following the treatment with ketamine 50 mg/kg and ketamine 12 mg/kg as compared to the vehicle.

A significant reduction of *D1R* gene expression was observed in the core of nucleus accumbens (**Core**, ANOVA, p=0.0474; F(2,8)=4.5739) following the treatment with ketamine as compared to the vehicle.

No significant changes in *D1R* expression were detected in anterior cingulate cortex (**AC**, ANOVA, p=0.1945; F(2,8)=2.0232), premotor cortex (**M2**, ANOVA, p=0.2749; 2,8=1.5240); motor cortex (**M1**, ANOVA, p=0.1067; F(2,8)=2.9984); somatosensory cortex (**SS**, ANOVA, p=0.0750; F(2,8)=3.6440); insular region (**I**, ANOVA, p=0.0819; F(2,8)=3.4763); dorsomedial caudate putamen (**DMCP**, ANOVA, p=0.1008; F(2,8)=3.0987); ventromedial caudate putamen (**VMCP**, ANOVA, p=0.0690; F(2,8)=3.8057); shell of accumbens (**Shell**, ANOVA, p=0.0856; F(2,8)=3.3953) following the treatment with ketamine 50 mg/kg or ketamine 12 mg/kg as compared to the vehicle.

D2R- Acute paradigm

Results, with respective ANOVA values and Student-Newman-Keuls post hoc test, autoradiographic images and graphics are shown in Fig.7-8.

No significant induction of *D2R* gene expression was observed in cortical subregions: in anterior cingulate cortex (**AC**, ANOVA, p=0.3874; F(2,8)=1.0700), premotor cortex (**M2**, ANOVA, p=0.0777; F(2,8)=3.5751); motor cortex (**M1**, ANOVA, p=0.3503; F(2,8)=1.1995); somatosensory cortex (**SS**, ANOVA, p=0.7187; F(2,8)=0.3443), insular region (**I**, ANOVA, p=0.4035; F(2,8)=1.0189) following the treatment with ketamine 50 mg/kg, ketamine 12 mg/kg as compared to the vehicle.

No significant induction of *D2R* gene expression was observed in striatum subregions: in dorsolateral caudate putamen (**DLCP**, ANOVA, p=0.3784; F(2,8)=1.0999), in ventrolateral caudate putamen (**VLCP**, ANOVA, p=0.2781; F(2,8)=1.5083); in dorsomedial caudate putamen (**DMCP**, ANOVA, p=0.2250; F(2,8)=1.8079); in ventromedial caudate putamen (**VMCP**, ANOVA, p=0.2288; F(2,8)=1.7837); following the treatment with ketamine 50 mg/kg or ketamine 12 mg/kg as compared to the vehicle.

No significant changes in *D2R* expression were detected in core of nucleus accumbens (**Core**, ANOVA, p=0.0888; F(2,8)=3.3269) and shell of accumbens (**Shell**, ANOVA, p=0.6072; F(2,8)=0.5314) following the treatment with ketamine 50 mg/kg or ketamine 12 mg/kg as compared to the vehicle.

No significant changes in *D2R* expression were detected in ventrotegmental Area (**VTA**, ANOVA, p=0.7017; F(2,8)=0.3703); substantia nigra pars compacta (**SNpc**, ANOVA, p=0.9862; F(2,8)=0.0139); substantia nigra pars reticulata (**SNpr**, ANOVA, p=0.7898; F(2,8)=0.2431) following the treatment with ketamine 50 mg/kg or ketamine 12 mg/kg as compared to the vehicle.

D1R- Subchronic paradigm

Results, with respective ANOVA values and Student's T test; graphics and autoradiographic images are shown in Fig.9.

No significant induction of *D1R* gene expression was observed in cortical subregions: in anterior cingulate cortex (**AC**, ANOVA, p=0.8260; F(2,4)=0.0528), premotor cortex (**M2**, ANOVA, p=0.5411; F(2,4)=0.4197); motor cortex (**M1**, ANOVA, p=0.8625; F(2,4)=0.0327); somatosensory cortex (**SS**, ANOVA, p=0.7528; F(2,4)=0.1087); insular region (**I**, ANOVA, p=0.7882; F(2,4)=0.0789) following the treatment with ketamine 12 mg/kg as compared to the vehicle.

No significant induction of *D1R* gene expression was observed in striatum subregions: in dorsolateral caudate putamen (**DLCP**, ANOVA, p=0.8555; F(2,4)=0.0362); ventrolateral caudate putamen (**VLCP**, ANOVA, p=0.7559; F(2,4)=0.1059); dorsomedial caudate putamen (**DMCP**, ANOVA, p=0.5117; F(2,4)=0.4862); ventromedial caudate putamen (**VMCP**, ANOVA, p=0.5594; F(2,4)=0.3818) following the treatment with ketamine 12 mg/kg as compared to the vehicle.

No significant changes in *D1R* expression were detected in core of nucleus accumbens (**Core**, ANOVA, p=0.6844; F(2,4)=0.1821) and shell of accumbens (**Shell**, ANOVA, p=0.8791; F(2,4)=0.0252) following the treatment with ketamine 12 mg/kg as compared to the vehicle.

D2R- Subchronic paradigm

Results, with respective ANOVA values and Student's t test, graphics and autoradiographic images are shown in Fig.10-11. A significant induction of *D2R* gene expression was observed in ventrotegmental area (**VTA**, ANOVA, p<0.0001; F(2,4)=133.9264) following the treatment with ketamine 12 mg/kg as compared to the vehicle. A significant induction of *D2R* gene expression was observed in retrosplenial dysgranular cortex (**RSCd**, ANOVA, p=0.0071; F(2,4)=16.0560) following the treatment with ketamine 12 mg/kg as compared to the vehicle.

No significant induction of *D2R* gene expression was observed in cortical subregions: in anterior cingulate cortex (**AC**, ANOVA, p=0.9909; F(2,4)=0.0001), premotor cortex (**M2**, ANOVA, p=0.7094; F(2,4)=0.1527); motor cortex (**M1**, ANOVA, p=0.6526; F(2,4)=0.2242); somatosensory cortex (**SS**, ANOVA, p=0.7687; F(2,4)=0.0947), insular region (**I**, ANOVA, p=0.9988; F(2,4)=0) following the treatment with ketamine 12 mg/kg as compared to the vehicle. No significant induction of *D2R* gene expression was observed in striatum subregions: dorsolateral caudate putamen (**DLCP**, ANOVA, p=0.7807; F(2,4)=0.0848); ventrolateral caudate putamen (**VLCP**, ANOVA, p=0.9882; F(2,4)=0.0002); dorsomedial caudate putamen (**DMCP**, ANOVA, p=0.9260; F(2,4)=0.0094); ventromedial caudate putamen (**VMCP**, ANOVA, p=0.9452; F(2,4)=0.0051) following the treatment with ketamine 12 mg/kg as compared to the vehicle. No significant changes in *D2R* expression were detected in core of nucleus accumbens (**Core**, ANOVA, p=0.9885; F(2,4)=0.0002) and shell of accumbens (**Shell**, ANOVA, p=0.5705; F(2,4)=0.3599) following the treatment with ketamine 12 mg/kg as compared to the vehicle. No significant changes in *D2R* expression were detected in substantia nigra pars compacta (**SNpc**, ANOVA, p=0.2462; F(2,4)=1.6512); substantia nigra pars reticulate (**SNpr**, ANOVA, p=0.1826; F(2,4)=2.2704); retrosplenial granular cortex (**RSCg**, ANOVA, p=0.0531; F(2,4)=5.7762) following the treatment with ketamine 12 mg/kg as compared to the vehicle.

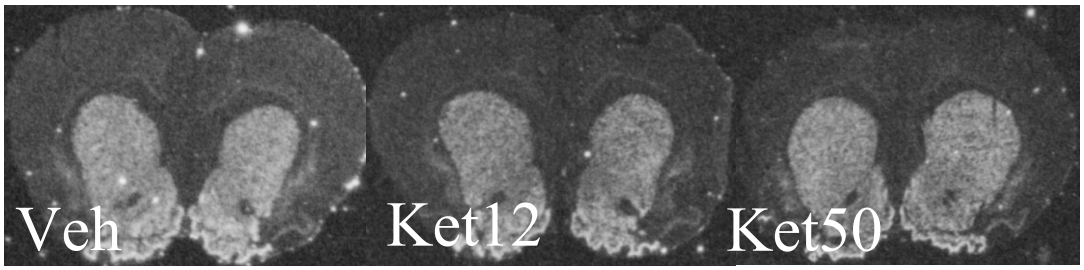
DAT- Subchronic paradigm

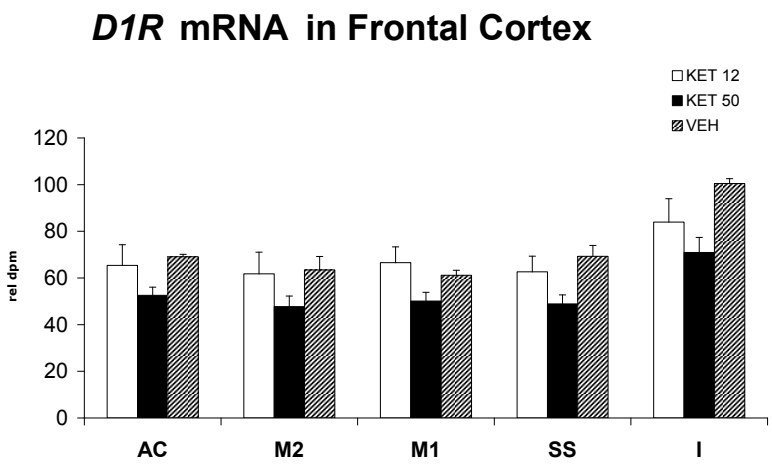
Results, with respective ANOVA values and Student's t test; graphics and autoradiographic images are shown in Fig.12. A significant induction of *DAT* gene expression was observed in substantia nigra pars compacta (**SNpc**, ANOVA, p=0.0189;

F(2,4)=10.1490); ventrotegmental area (**VTA**, ANOVA, p=0.0419; F(2,4)=6.6401) following the treatment with ketamine 12 mg/kg as compared to the vehicle.

A significant reduction of *DAT* gene expression was observed in retrosplenial granular cortex (**RSCg**, ANOVA, p=0.0231; F(2,4)=10.4565); retrosplenial dysgranular cortex (**RSCd**, ANOVA, p=0.0081; F(2,4)=18.0824) following the treatment with ketamine 12 mg/kg as compared to the vehicle.

No significant changes in *DAT* expression were detected in substantia nigra pars reticulata (**SNpr**, ANOVA, p=0.3485; F(2,4)=1.0340); following the treatment with ketamine 12 mg/kg as compared to the vehicle.





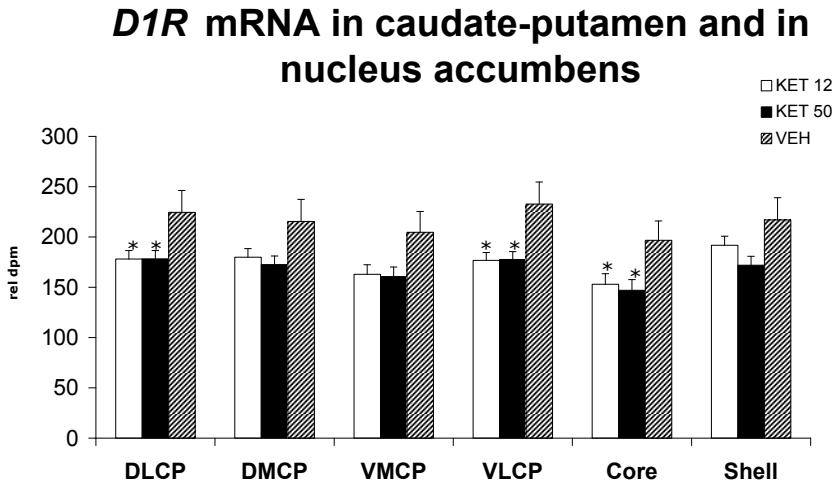
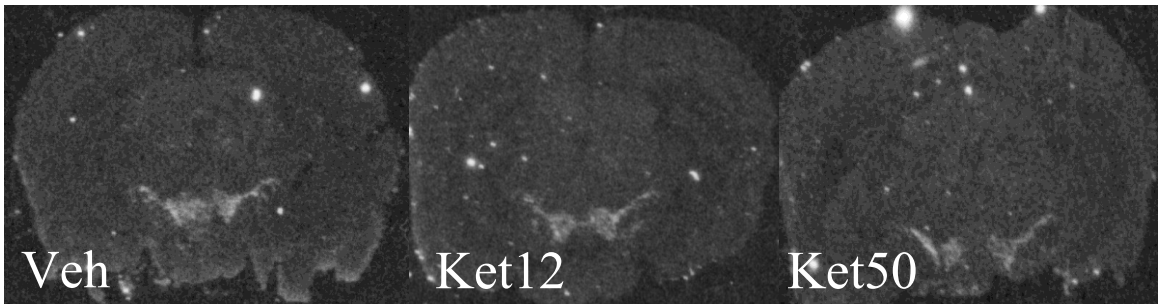


Figure 6. Autoradiographic image and graphic of *D1R* mRNA expression in Frontal Cortex, Caudate Putamen and Nucleus Accumbens expressed in relative dpm as mean ± S.E.M.; *ANOVA $p<0,05$ vs VEH.



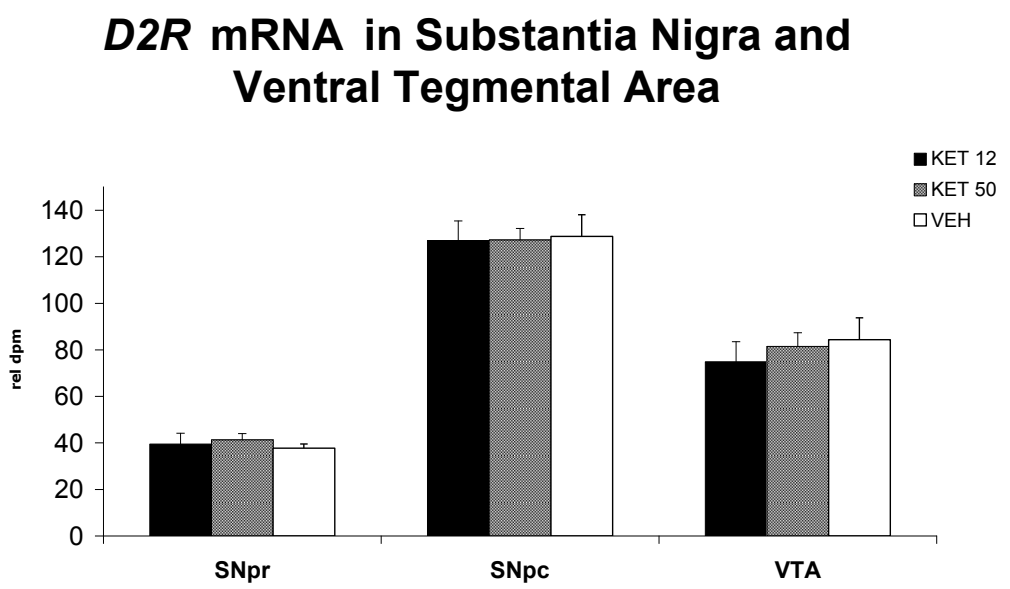
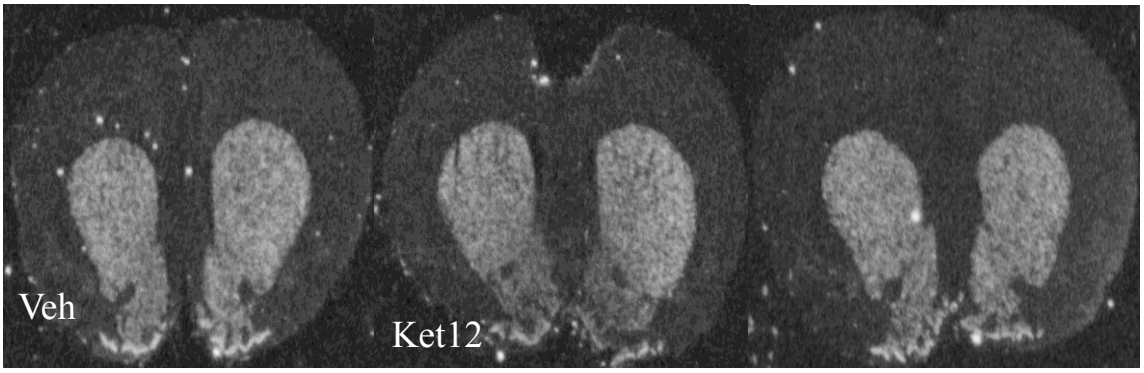
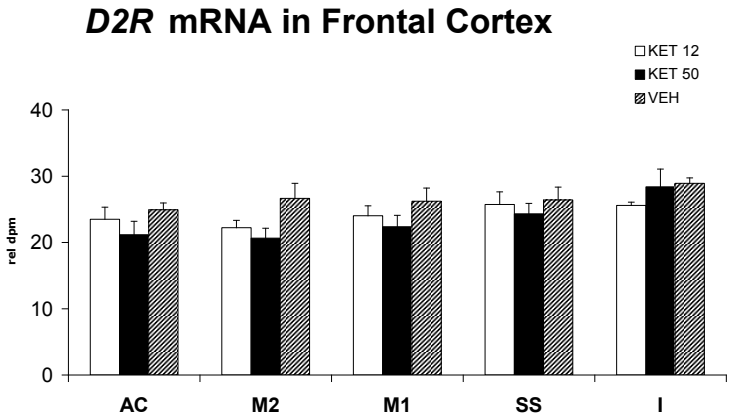


Figure 7. Autoradiographic image and graphic of *D2R* mRNA expression in Substatia Nigra and Ventrotegmental Area expressed in relative dpm as mean ± S.E.M.; *ANOVA $p<0,05$ vs VEH.





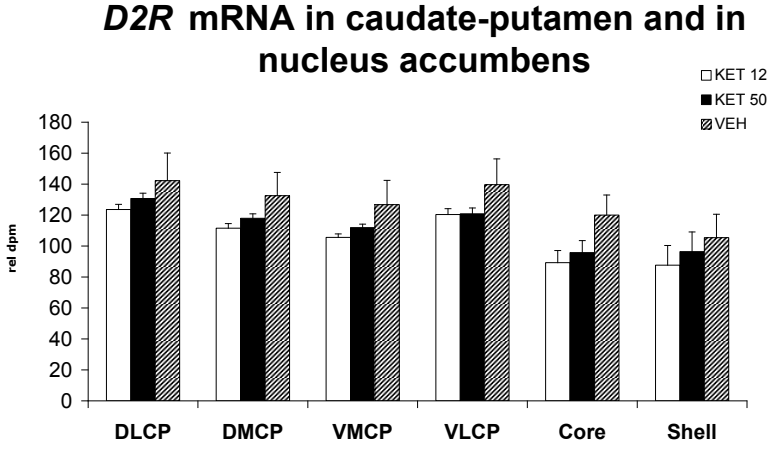
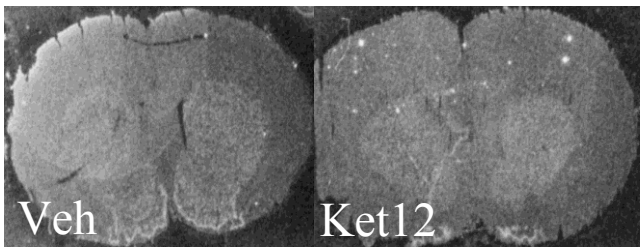
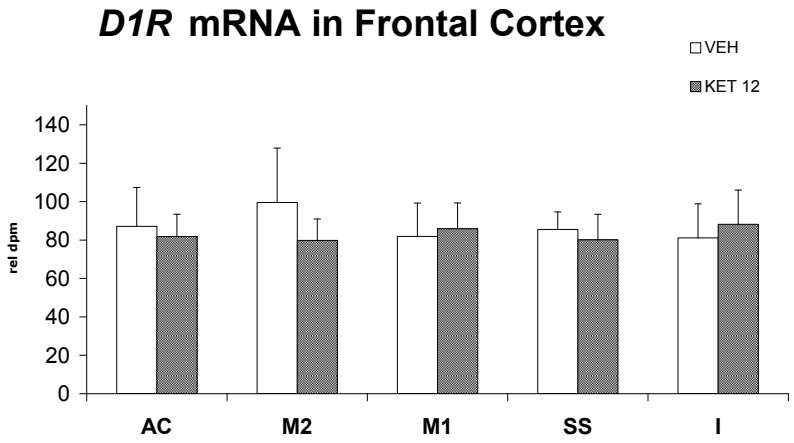


Figure 8. Autoradiographic images and graphic of *D2R* mRNA expression in Frontal Cortex, Caudate Putamen and Nucleus Accumbens expressed in relative dpm as mean ± S.E.M.; *ANOVA $p<0,05$ vs VEH.





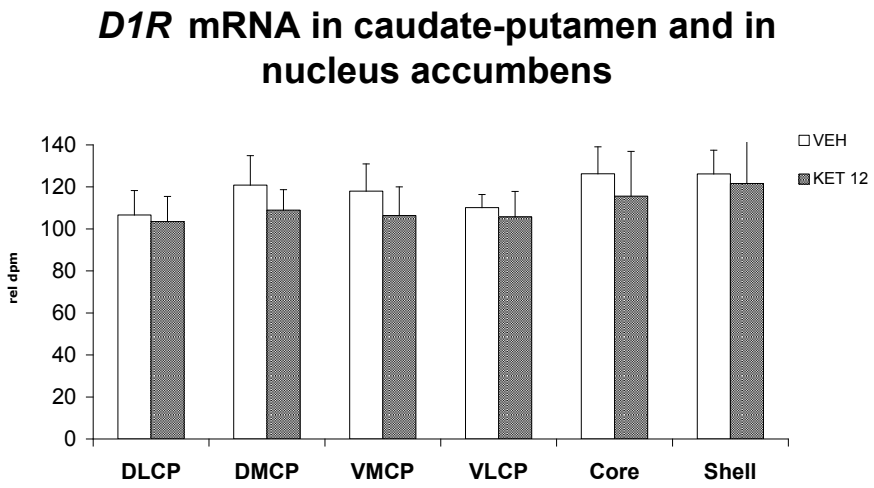
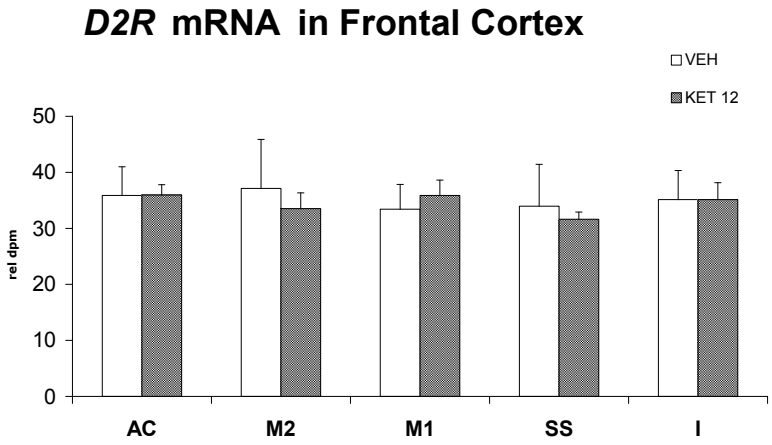


Figure 9. Autoradiographic images and graphic of *D1R* mRNA expression in Frontal Cortex, Caudate Putamen and Nucleus Accumbens expressed in relative dpm as mean ± S.E.M.; *ANOVA $p<0,05$ vs VEH.



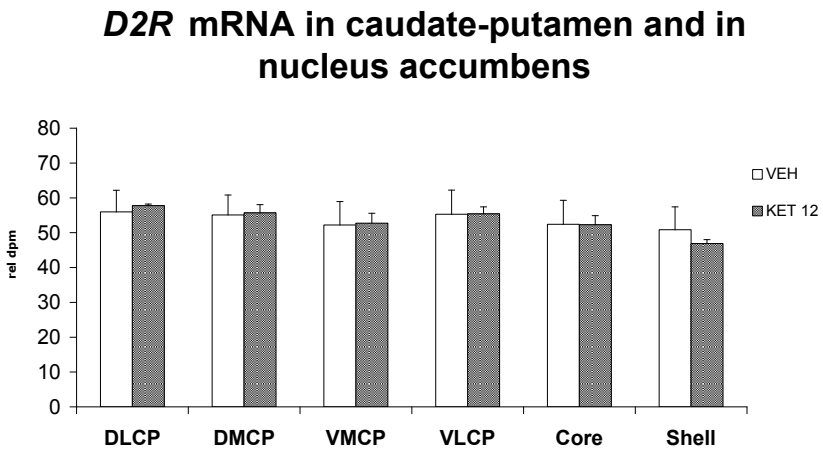
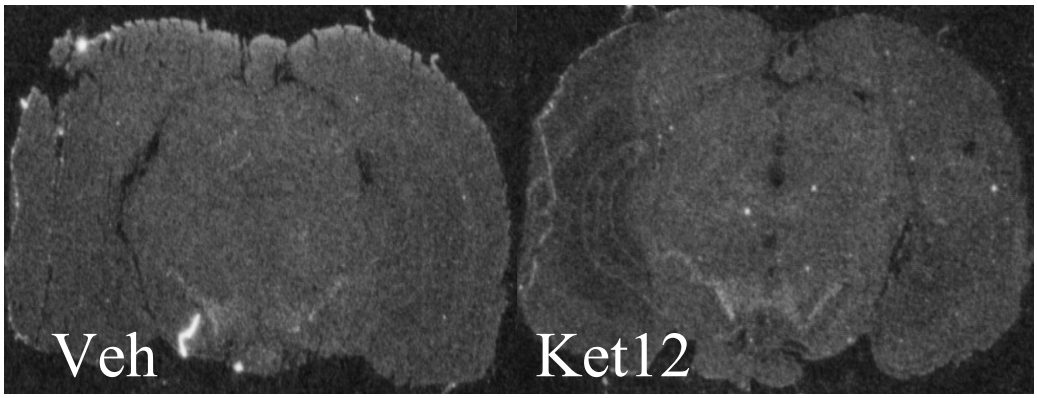


Figure 10. Autoradiographic image and graphic of *D2R* mRNA expression in Frontal Cortex, Caudate Putamen and in Nucleus Accumbens expressed in relative dpm as mean ± S.E.M.; *ANOVA $p<0,05$ vs VEH.



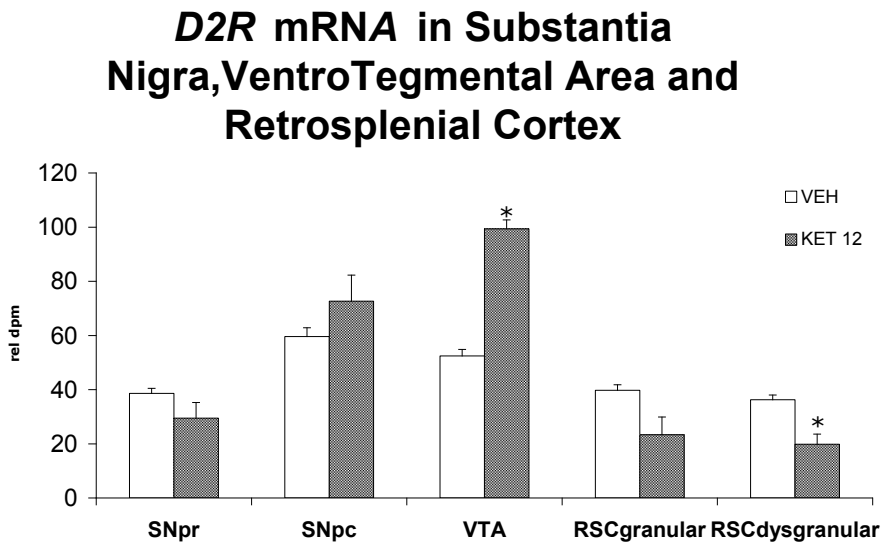
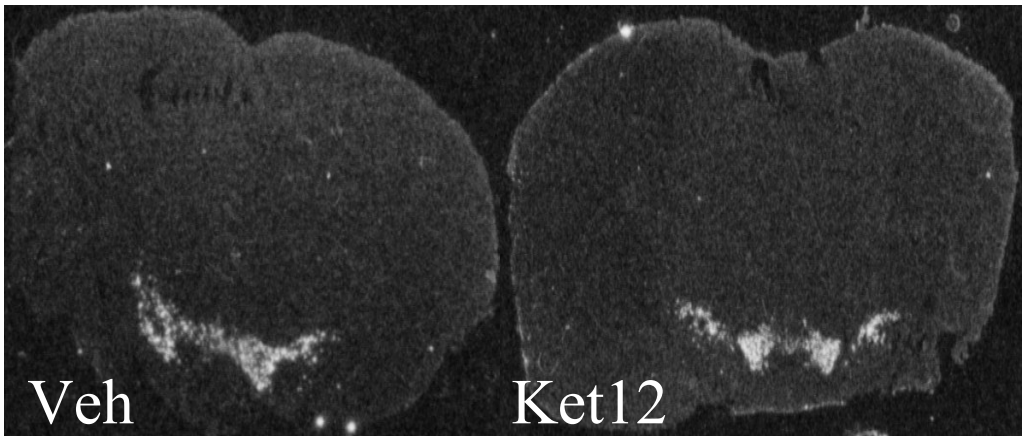


Figure 11. Autoradiographic image and graphic of *D2R* mRNA expression in Substantia Nigra, Ventrotegmental Area expressed in relative dpm as mean ± S.E.M.; *ANOVA $p<0,05$ vs VEH.



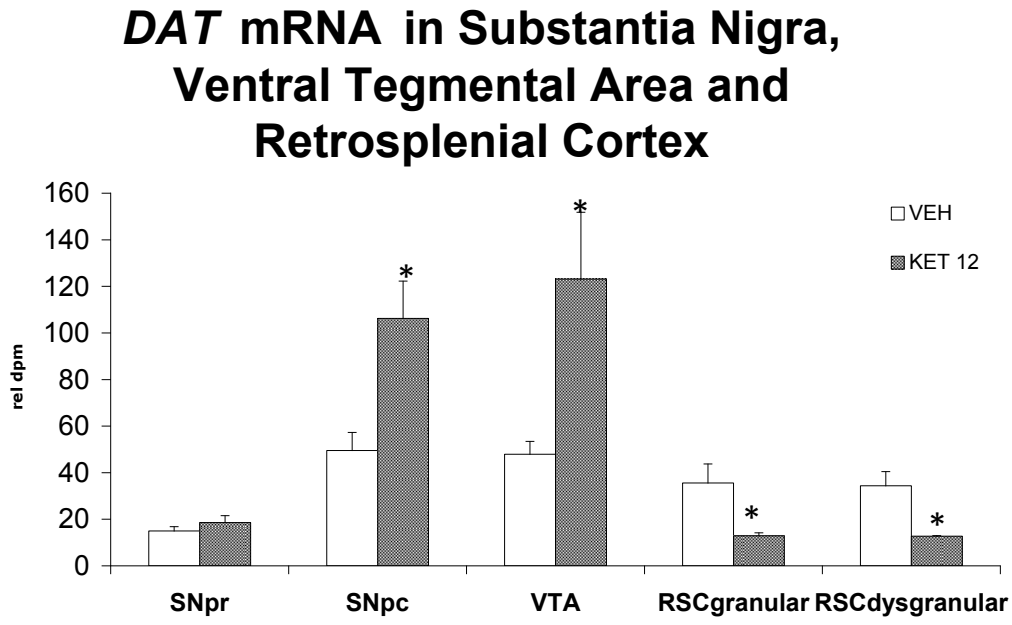


Figure 12. Autoradiographic image and graphic of *DAT* mRNA expression in Substantia Nigra, Ventrotegmental Area and Retrosplenial Cortex expressed in relative dpm as mean ± S.E.M.; *ANOVA $p<0,05$ vs VEH.

Chapter 5.

Modulation of constitutive and inducible early genes implicated in glutamate neurotransmission by different antagonists at NMDA receptors.

Rationale.

Dysfunctions in glutamatergic neurotransmission have been considered one of the main mechanisms in the pathophysiology of psychosis. Administration of non-competitive blockers of NMDA-R (i.e.: PCP, ketamine, and MK-801) has been observed to exacerbate psychotic symptoms in schizophrenic individuals and to trigger an exogenous psychosis in healthy volunteers recalling positive and negative symptoms of schizophrenia (Javitt and Zukin 1991; Krystal et al. 1994; Adler et al. 1999; Heresco-Levy 2003; Krystal et al. 2003; Javitt 2010). Exposure to these compounds has been described to cause behavioral manifestations in rodents mimicking positive, negative, and cognitive features of schizophrenia (Carlsson and Svensson 1990; Tiedtke et al. 1990; Riederer et al. 1991; Schmidt et al. 1991) and to trigger neuropathological changes that may represent the anatomic underpinnings of psychosis (Olney et al. 1999). This body of evidence has led to the formulation of the NMDA-R hypofunction (NRH) hypothesis of psychosis pathophysiology (Olney et al. 1999). NRH hypothesis predicts that a condition of NRH may induce complex dysfunctions in several neurotransmitter systems at both cortical and subcortical levels leading to information process failure and ultimately to psychotic symptoms (Moghaddam et al. 1997; Olney et al. 1999; Farber 2003).

Administration of antagonists at NMDA-Rs has been regarded as a valuable and heuristic animal model of psychosis (Lipska and Weinberger 2000). This pharmacological model is considered to have good predictive and construct validity

(Lipska and Weinberger 2000), allowing to identify new therapeutic targets and to study putative molecular dysfunctions implicated in psychosis.

According to the view that glutamatergic transmission may be involved in psychosis pathophysiology, accumulating evidence is reporting the association of glutamatergic molecules to preclinical or clinical models of psychosis. Genes implicated in glutamate neurotransmission have been consistently associated to schizophrenia (de Bartolomeis et al. 2005). Mice carrying a deficit mutation of the NMDA-R NR1 subunit displayed behavioral abnormalities similar to those observed in animal models of schizophrenia (Mohn et al. 1999). A reduction in NR1 subunit phosphorylation has been found in a subset of schizophrenic subjects (Li et al. 2009). Moreover, it has been observed that antipsychotics may alter gene expression and protein levels of molecules involved in glutamate transmission, as ionotropic glutamate receptors (Meador-Woodruff et al. 1996; Riva et al. 1997) or molecules of the PSD, as Homer. The PSD is a complex protein mesh located at the dendritic spines of post-glutamatergic neurons (de Bartolomeis et al. 2005), that is implicated in synaptic signal transduction, neurotransmitters interplay, and in the regulation of ultrastructural architecture and functional organization of spines (de Bartolomeis et al. 2005). In recent studies, the inducible isoform of *Homer1* gene resulted to be expressed after acute and chronic antipsychotic administration (Iasevoli et al. 2010; Iasevoli et al. 2010). *Homer1a* expression, indeed, was found to be triggered by selective antagonism on dopamine D2Rs (Iasevoli et al. 2009) as well as by ketamine administration (Iasevoli et al. 2007), suggesting that the pattern of *Homer1a* expression may be a powerful marker of glutamatergic and dopaminergic function and of their interplay.

Recently, compounds acting on glutamatergic neurotransmission have been proposed and tested in the therapy of psychosis. Memantine is a derivative of amantadine acting

as NMDA-R partial uncompetitive antagonist (Johnson and Kotermanski 2006; Parsons et al. 2007). Memantine has been approved for clinical use in Alzheimer's disease. However, the drug is under investigation for use in several other conditions, including the use as add-on to antipsychotics in the treatment of refractory schizophrenia and for the improvement of cognitive failure in psychosis (Parsons et al. 2007; Zdanys and Tampi 2008).

These clinical properties may derive from the peculiar pharmacological action of memantine. Memantine binds NMDA-R in a use-dependent and voltage-dependent way (Johnson and Kotermanski 2006; Parsons et al. 2007) in M2 region of NR1 and NR2 subunits. Memantine acts as a partial trapping blocker, probably because there are two binding sites in NMDA-R: a deep site inside the external gate that allows full trapping, and a second shallow site outside the gate that may lead to a partial inhibition of channel closure, increasing channel in open state. Ketamine and MK-801 bind to the deep site, probably for this reason they have different profile compared to memantine (Johnson and Kotermanski 2006; Parsons et al. 2007).

Under resting potential, -70mV, Mg++, memantine and MK-801 bind the NMDA-R. Both Mg++ and memantine may leave the channel upon strong depolarization, i.e.: -20 mV, whereas MK-801 remains trapped. Memantine compared to Mg++ does not leave the channel upon moderate prolonged depolarization, i.e.: -50 mV, during chronic excitotoxic stimulus, as that induced by β amyloid (Parsons et al. 2007). While high affinity blockers, such as MK-801, block the pathological and physiological activation of NMDA-R, memantine decreases synaptic noise resulting from excessive NMDA-R activation, allows the physiological synaptic signaling and re-balances between inhibition and excitation of glutamate system (Parsons et al. 1999; Parsons et al. 2007; Gilling et al. 2009). Memantine prolongs duration of post-synaptic NMDA-R-

dependent Long-Term Potentation (LTP), important for neuronal memory (Johnson and Kotermanski 2006; Parsons et al. 2007).

Moreover, memantine behaves as a dopamine D2R agonist that induces dopamine release in rat prefrontal cortex and striatum and reduces dopamine outflow in the hippocampus (Spanagel et al. 1994; Andreassen et al. 1996; Hesselink et al. 1999; Peeters et al. 2003; Giustizieri et al. 2007; Meisner et al. 2008; Seeman et al. 2008).

Memantine upregulates mRNA and protein expression of the brain-derived neurotrophic factor (BDNF), and inhibits gene expression of Heat Shock Protein (HSP70) in hypoxic ischemia as protective effect (Caumont et al. 2006; Rosi et al. 2006; Shearman et al. 2006). Indeed memantine reduces the rates of apoptosis in neuronal hypoxemic damage in the hippocampus and in the striatum (Chen et al. 1992; Block and Schwarz 1996; Ehrlich et al. 1999).

In behavioral studies memantine does not induce hyperlocomotion at 5 mg/kg, while 10 and 25 mg/kg doses cause small sustained increase in locomotion, as ketamine (Eisenberg et al. 1993; Gilmour 2009). At active lever pressing test memantine, as ketamine, decreases instrumental output with a dose-dependent effect (Gilmour 2009). In water maze test, memantine has been observed to improve hippocampus- based spatial learning in a transgenic mouse model of Alzheimer's Disease (Minkeviciene et al. 2004).

Memantine may thus improve cognitive functions and may reveal beneficial in psychosis. However, memantine acts primarily on the same binding target of ketamine and MK-801, two compounds known to induce psychosis and used to model psychosis in animals (Bubenikova-Valesova et al. 2008). The aim of this study is to evaluate whether behaviorally active doses of memantine, ketamine, and MK-801 trigger changes in the gene expression of molecules of the PSD implicated in glutamate

signaling and dendrite functional status. Moreover, we wanted to verify whether putative molecular changes are similar or discrepant among the compounds tested, as to test the hypothesis that differences in behavioral outcomes among them may derive from the induction of different molecular responses, albeit acting on the same biological target.

Results.

Arc

Results, with respective ANOVA values and Student-Newman-Keuls post hoc test, are detailed in Table 3; autoradiographic images are shown in Fig.13. A significant induction of *Arc* gene expression was observed in the anterior cingulate cortex (**AC**, ANOVA, $p=0.0011$; $F(4,18)=8.1157$); the premotor cortex (**M2**, ANOVA, $p<0.0001$; $F(4,18)=13.6178$); the motor cortex (**M1**, ANOVA, $p<0.0001$; $F(4,18)=15.3103$); the somatosensory cortex (**SS**, ANOVA, $p<0.0001$; $F(4,17)=19.2305$) and in the insular region (**I**, ANOVA, $p<0.0001$; $F(4,17)=17.6433$) following the treatment with memantine 5 mg/kg, MK-801 0.8 mg/kg, ketamine 25 mg/kg, ketamine 50 mg/kg, as compared to the vehicle.

A significant induction of *Arc* gene expression was observed in the ventrolateral caudate putamen (**VLCP**, ANOVA, $p=0.0288$; $F(4,16)=3.8229$); in ventromedial caudate putamen (VMCP, ANOVA, $p=0.0321$; $F(4,16)=3,6908$); in the core of accumbens (**Core**, ANOVA, $p=0.0026$; $F(4,16)=7.3206$) following the treatment with ketamine 50 mg/kg and MK-801 0.8 mg/kg as compared to the vehicle and in the shell of accumbens (**Shell**, ANOVA, $p=0.0007$; $F(4,16)=9.7566$) following the treatment with MK-801 0.8 mg/ kg, ketamine 50 mg/kg and ketamine 25 mg/kg as compared to the vehicle.

No significant changes in *Arc* expression were detected in dorsolateral caudate putamen (**DLCP**, ANOVA, $p=0.1261$; $F(4,16)=2.1665$) and in dorsomedial caudate putamen (**DMCP**, ANOVA, $p=0.1170$; $F(4,16)=2.2399$).

c-fos

Results, with respective ANOVA values and Student-Newman-Keuls post hoc test, are detailed in Table 4; autoradiographic images are shown in Fig.14. A significant induction

of *c-fos* gene expression was observed in the anterior cingulate cortex (**AC**, ANOVA, p=0.0082; F(4,16)=5.7201) following the treatment with MK-801 0.8 mg/kg; in the premotor cortex (**M2**, ANOVA, p=0.0026; F(4,16)=7.3356) following the treatment with MK-801 0.8 mg/kg, ketamine 50 mg/kg and memantine 5 mg/kg, as compared to the vehicle; in the insular region (**I**, ANOVA, p<0.0001; F(4,16)=39.8114) following the treatment with MK-801 0.8 mg/kg, ketamine 25 mg/kg, memantine 5 mg/kg and ketamine 50 mg/kg as compared to the vehicle. The treatment with ketamine 50 mg/kg significantly decreased *c-fos* expression as compared to the vehicle in the dorsolateral caudate putamen (**DLCP**, ANOVA, p=0.0104; F(4,16): 5.1450). In the ventrolateral caudate-putamen (**VLCP**, ANOVA, p=0.0025; F(4,16): 7.0750), the treatment with ketamine 50 mg/kg significantly decreased *c-fos* expression as compared to the vehicle, while the treatment with MK-801 0.8 mg/kg increased *c-fos* expression as compared to the vehicle.

No significant changes in *c-fos* expression were detected in motor cortex (**M1**, ANOVA, p=0.1922; F(4,16)=1.7840), somatosensory cortex (**SS**, ANOVA, p=02750; F(4,16)=1.444); dorsomedial caudate putamen (**DMCP**, ANOVA, p=0.2139; F(4,16)=1.6814); ventromedial caudate putamen (**VMCP**, ANOVA, p=0.0560; F(4,16)=2.9908) and in nucleus accumbens (**Core**, ANOVA, p=0.0707; F(4,16)=2.7468; **Shell**, ANOVA, p=0.0322; F(4,16)=3.5977).

Homer 1a

Results, with respective ANOVA values and Student-Newman-Keuls post hoc test are detailed in Table 5; autoradiographic images are shown in Fig.15. *Homer 1a* gene expression was found decreased in the motor cortex (**M1**, ANOVA, p=0.0311; F(4,16)=3.7306); in somatosensory cortex (**SS**, ANOVA, p=0.0023; F(4,16)=7.5474)

following the treatment with memantine 5 mg/kg and ketamine 50 mg/kg as compared to the vehicle. *Homer 1a* increased following the treatment with memantine 5 mg/kg as compared to the vehicle, and decreased following the treatment with ketamine 25 mg/kg as compared to the vehicle; in insular region (**I**, ANOVA, p=0.0155; F(4,16)=4.6095) following the treatment with ketamine 25 mg/kg, as compared to the vehicle.

No significant changes in *Homer 1a* expression were detected in anterior cingulate cortex (**AC**, ANOVA, p=0.0411; F(4,16)=3.4027), premotor cortex (**M2**, ANOVA, p=0.1433; F(4,16)=2.0706); dorsolateral caudate putamen (**DLCP**, ANOVA, p=0.1371; F(4,16)=2.1490); dorsomedial caudate putamen (**DMCP**, ANOVA, p=0.0870; F(4,16)=2.5783); ventromedial caudate putamen (**VMCP**, ANOVA, p=0.2279; F(4,16)=1.6214); ventrolateral caudateputamen (**VLCP**, ANOVA, p=0.02070; F(4,16)=4.2348) and in nucleus accumbens (**Core**, ANOVA, p=0.0832; F(4,16)=2.6253; **Shell**, ANOVA, p=0.3921; F(4,16)=1.1126).

Homer 1b

Results, with respective ANOVA values and Student-Newman-Keuls post hoc test are detailed in Table 6; autoradiographic images are shown in Fig.16. *Homer 1b* gene expression was found decreased in premotor cortex (**M2**, ANOVA, p=0.0265; F(4,16)=3.7385), following the treatment with ketamine 50 mg/kg as compared to the vehicle; in motor cortex (**M1**, ANOVA, p=0.0023; F(4,16)=6.9258) following the treatment with ketamine 25 mg/kg and ketamine 50 mg/kg, as compared to the vehicle. In the dorsolateral caudate putamen (**DLCP**, ANOVA, p=0.0249, F(4,17)=3.7345) *Homer 1b* gene expression was decreased by the treatment with ketamine 25 mg/kg and 50 mg/kg as compared to the vehicle.

No significant changes in *Homer 1b* expression were detected in anterior cingulate cortex (**AC**, ANOVA, p=0.2988; F(4,16)=1.3459); somatosensory cortex (**SS**, ANOVA, p=0.1122; F(4,17)=2.2249); insular region (**I**, ANOVA, p=0.0680; F(4,17)=2.7019); dorsomedial caudate putamen (**DMCP**, ANOVA, p=0.3897; F(4,17)=1.1012); ventromedial caudate putamen (**VMCP**, ANOVA, p=0.4963; F(4,17)=0.8826); ventrolateral caudate putamen (**VLCP**, ANOVA, p=0.3642; F(4,17)=1.1617) and in nucleus accumbens (**Core**, ANOVA, p=0.2058; F(4,17)=1.6705; **Shell**, ANOVA, p=0.9905; F(4,17)=0.0688).

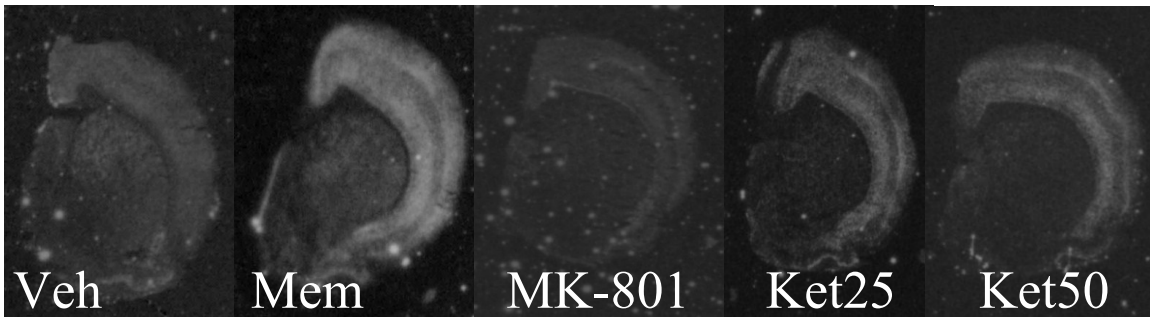
PSD95

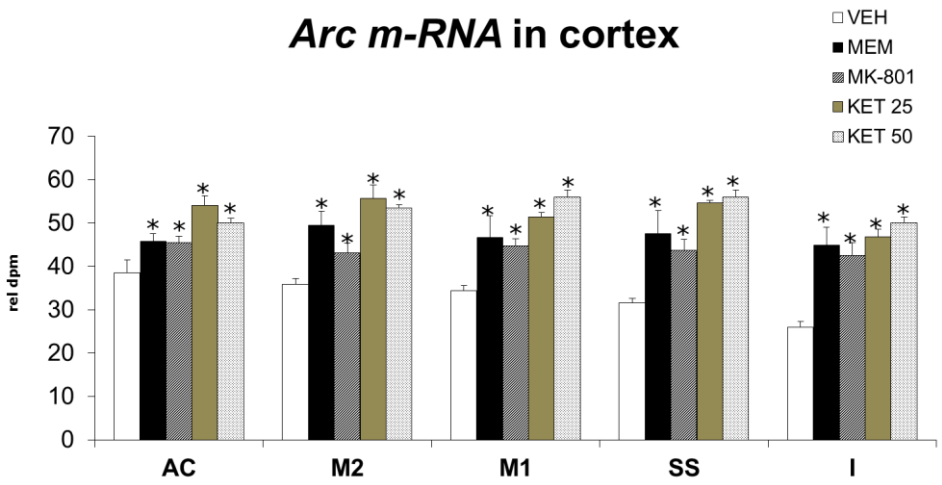
Results, with respective ANOVA values and Student-Newman-Keuls post hoc test are detailed in Table 7; autoradiographic images are shown in Fig.17. A significant decrease of *PSD95* gene expression was observed in the dorsomedial caudate putamen (**DMCP**, ANOVA, p=0.0270; F(4,15)=3.8006) following the treatment with ketamine 25 mg/kg, ketamine 50 mg/kg, MK-801 0.8 mg/kg, as compared to the vehicle; in the dorsolateral caudate putamen (**DLCP**, ANOVA, p=0.0158; F(4,15)=4.4457) and in the .entromedial caudate putamen (**VMCP**, ANOVA, p=0.0431; F(4,15)=3.2747) following the treatment with MK-801 0.8 mg/kg, as compared to the vehicle.

No significant changes in *PSD95* expression were detected in anterior cingulate cortex (**AC**, ANOVA, p=0.2119; F(4,15)=1.6903); premotor cortex (**M2**, ANOVA, p=0.0682; F(4,15)=2.8369); motor cortex (**M1**, ANOVA, p=0.5807; F(4,15)=0.7411); somatosensory cortex (**SS**, ANOVA, p=0.0448; F(4,15)=3.2306); insular region (**I**, ANOVA, p=0.4740; F(4,15)=0.9311); ventrolateral caudate putamen (**VLCP,** ANOVA, p=0.5887; F(4,15)=0.7261); and in nucleus accumbens (**Core**, ANOVA, p=0.1326; F(4,15)=2.1179; **Shell**, ANOVA, p=0.2995; F(4,15)=1.3638).

Arc	Memantine	MK-801	Ket 50	Ket 25	Veh	ANOVA (p, F)
Frontal cortex						
Anterior Cingulate cortex	45.7057± 1.8611*	45.5374± 1.4107*	50.0785± 1.0328*	53.9778± 2.3036*	38.5084± 3.0334	***p = 0.0011***, $F_{4.15} = 8.1157$
Medial Agranular Cortex	49.4633± 3.3249*	43.1678± 2.1047*	53.5718± 0.6293*	55.7157± 3.0262*	35,8497± 1,4005	***p < 0.0001***, $F_{4.15} = 13.6178$
Motor Cortex	46.6217± 5.0033*	44.8029± 1.5637*	56.1015± 1.5651*	51.4047± 1.0699*	34,3722± 1,2674	***p < 0.0001***, $F_{4.15} = 15.3103$
Somatosensory Cortex	47.4757± 5.3192*	43.7932± 2.4391*	56.0727± 1.4725*	54.6354± 0.5926*	31,6188± 0,9341	***p < 0.0001***, $F_{4.14} = 9.2305$
Insular Cortex	44.9480± 4.0336*	42.5406± 2.9450*	50.1640± 1.2240*	46.7972± 1.7877*	26,0336± 1,3166	***p < 0.0001***, $F_{4.14} = 7.6433$
Striatum						
Dorsomedial Caudate Putamen	31,1330± 3,6284	36,4460± 1,5252	38,0892± 1,0713	33,5373± 2,2610	32,0128± 1,2751	*p > 0.05,* $F_{4.14} = 2.1665$
Dorsolateral Caudate Putamen	32,6113± 4,2183	35,9424± 1,2429	36,5855± 0,9622	29,3330± 2,8426	31,1127± 1,1421	*p > 0.05,* $F_{4.14} = 2.2399$
Ventromedial Caudate Putamen	32,6187± 3,6894	36.7593± 0.8953*	36.2430± 0.8554*	29,4750± 1,9739	28,6124± 1,8427	***p = 0.0321***, $F_{4.13} = 3.6908$
Vetrolateral Caudate Putamen	32,7287± 3,7792	35.8753± 1.0140*	34.9005± 1.1229*	27,1395± 3,1993	26,2960± 1,1554	***p = 0.0288***, $F_{4.13} = 3.8229$
Core of the Nucleus Accumbens	25,4760± 2,8837	34.6110± 0.2733*	35.5977± 1.6234*	24,5585± 3,4390	21,2612± 1,9769	***p = 0.0026***, $F_{4.13} = 7.3206$
Shell of the Nucleus Accumbens	24,6173± 1,5475	35.3390± 1.0944*	34.3397± 0.7047*	27.1208± 3.8803*	19,2241± 1,0891	***p = 0.0007***, $F_{4.13} = 9.7566$

Table 3. mRNA levels of *Arc* after acute ketamine treatment. Data are expressed as d.p.m. mean values±standard error means (S.E.M.) and listed by brain regions analyzed, along with the relative ANOVA (degrees of freedom and p values). Significant p values are expressed in bold. Post-hoc test: * significantly higher expression compared to VEH.





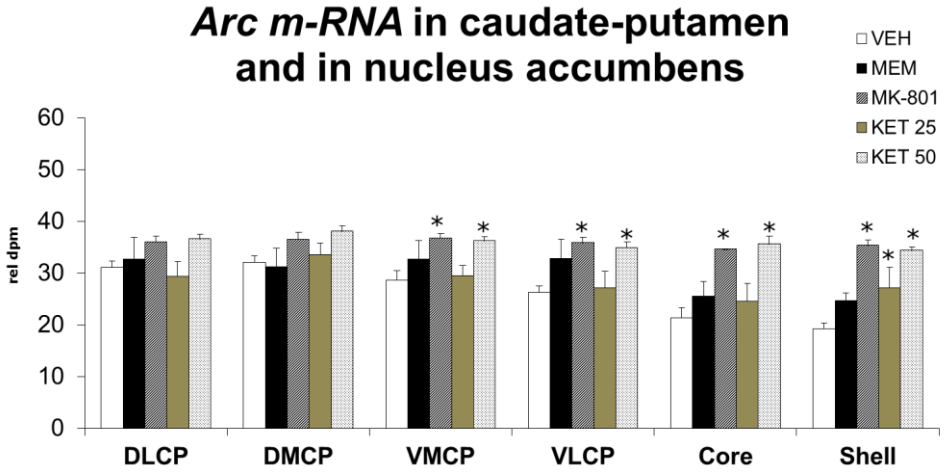
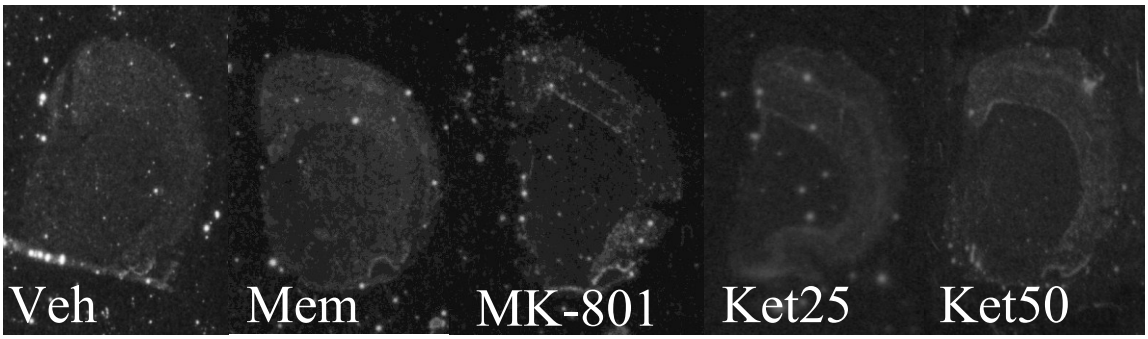
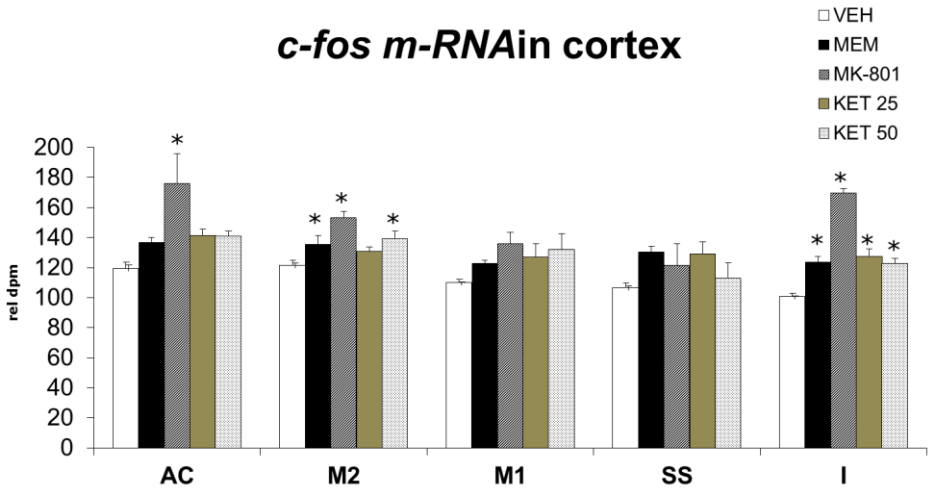


Figure 13. Autoradiographic image and graphics of *Arc* mRNA expression in Frontal Cortex, Striatum, Nucleus Accumbens expressed in relative dpm as mean ± S.E.M.; *ANOVA $p<0,05$ vs VEH.

c-fos	Memantine	MK-801	Ket 50	Ket 25	Veh	ANOVA (p, F)
Frontal cortex						
Anterior Cingulate cortex	136,606± 3,464	175.985± 19.983*	140,892± 3,339	139.445± 4.7039	119,714± 4,092	***p = 0.0082***, $F_{4.12} = 5.7201$
Medial Agranular Cortex	135.413± 5.7543*	153.261± 3.9777*	139,445± 4,7039*	130,578± 2,9979	121,511± 3,7471	***p = 0.0026*****,** $F_{4.13} = 7.3356$
Motor Cortex	122,552± 2,166	135,821± 7,738	132,064± 10,386	126,965± 8,819	110,111± 2,320	*p > 0.05,* $F_{4.13} = 1.7840$
Somatosensory Cortex	130,236± 3,963	121,820± 14,100	113,156± 10,133	128,962± 8,061	107,005± 2,725	*p > 0.05,* $F_{4.13} = 1.4440$
Insular Cortex	123.603± 3.8190*	169.713± 2.7242*	122.694± 3.3360*	127.268± 5.3554*	100,911± 2,3835	***p < 0.0001***, $F_{4.13} = 9.8114$
Striatum						
Dorsomedial Caudate Putamen	102,387± 3,829	106,756± 10,493	80,484± 8,450	90,304± 10,337	97,785± 2,623	***p = 0.0104***, $F_{4.13} = 5.1450$
Dorsolateral Caudate Putamen	101,963± 5,3429	100,988± 8,1072	70,665± 7,9377*	77,892± 6,0444	94,021± 1,7614	*p > 0.05,* $F_{4.13} = 1.6814$
Ventromedial Caudate Putamen	98,237± 4,426	107,766± 10,070	77,536± 7,560	82,560± 7,792	96,511± 1,870	*p > 0.05,* $F_{4.14} = 2.9908$
Vetrolateral Caudate Putamen	101,299± 6,3708	108.627± 8.2715*	65.746± 7.3540*	78,777± 6,7156	89,150± 0,2270	***p = 0.0025***, $F_{4.14} = 7.0750$
Core of the Nucleus Accumbens	98,425± 2,970	108,824± 3,564	75,013± 7,997	85,932± 9,262	91,798± 10,223	*p > 0.05,* $F_{4.14} = 2.7468$
Shell of the Nucleus Accumbens	100,714± 8,4896	119,155± 8,1766	76,154± 9,2383	87,611± 8,1134	99,219± 8,8841	*p > 0.05,* $F_{4.14} = 3.5977$

Table 4. mRNA levels of *c-fos* after acute ketamine treatment. Data are expressed as d.p.m. mean values±standard error means (S.E.M.) and listed by brain regions analyzed, along with the relative ANOVA (degrees of freedom and p values). Significant p values are expressed in bold. Post-hoc test: * significantly higher expression compared to VEH.





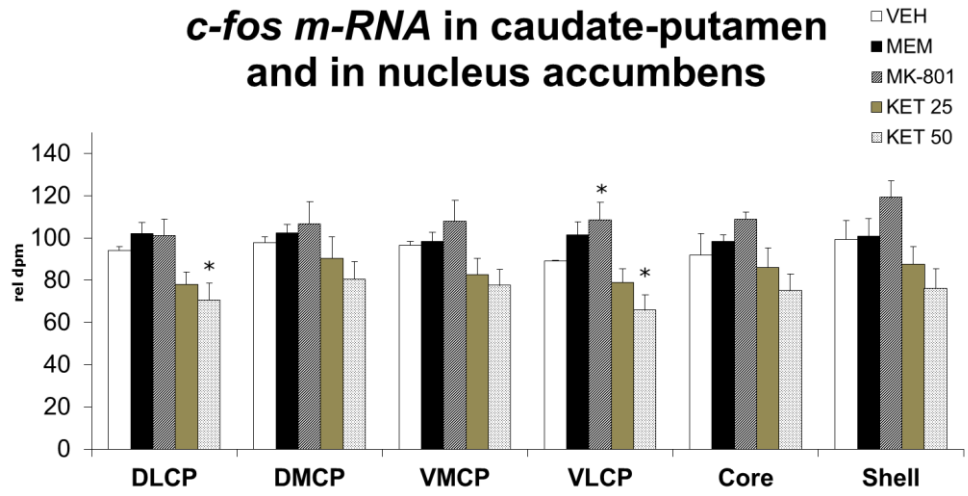


Figure 14. Autoradiographic image and graphics of *c-fos* mRNA expression in Frontal Cortex, Striatum, Nucleus Accumbens expressed in relative dpm as mean ± S.E.M.; *ANOVA p<0,05 vs VEH.

Homer1a	**Memantine**	**MK-801**	**Ket 50**	**Ket 25**	**Veh**	**ANOVA (p, F)**
Frontal cortex						
Anterior Cingulate cortex	72,7529± 4,2285	72,3036± 0,6897	59,0510± 3,4561	85,7285± 7,3110	74,6634± 6,3213	***p = 0,0411***, $F_{4.13}$ = 3,4027
Medial Agranular Cortex	64,5386± 6,6267	77,3206± 6,5160	63,5037± 3,1073	81,4304± 5,7097	79,5713± 7,5011	*p > 0.05*, $F_{4.13}$ = 2,0706
Motor Cortex	67,7075± 7,1902*	79,6898± 3,3459	72,2832± 4,4806*	87,9380± 3,8759	86,4840± 3,7012	***p = 0,0311***, $F_{4.13}$ = 3,7306
Somatosensory Cortex	62,1118± 3,4920*	71,3270± 3,5840	64,8863± 2,1594	82,1247± 2,4296*	72,8547± 3,0079	***p = 0,0023***, $F_{4.13}$ = 7,5474
Insular Cortex	73,3346± 7,8146*	74,5933± 3,1371	69,2346± 2,2896	87,5793± 1,3393*	66,0218± 4,6099	***p = 0,0155***, $F_{4.13}$ = 4,6095
Striatum						
Dorsomedial Caudate Putamen	54,8060± 4,4133	60,0616± 0,1218	50,9906± 4,1770	67,2087± 3,6108	59,2727± 4,5427	*p > 0.05*, $F_{4.13}$ = 2,5783
Dorsolateral Caudate Putamen	41,2143± 13,902	57,0164± 1,194	48,3230± 3,281	65,5899± 5,709	58,3051± 2,515	*p > 0.05*, $F_{4.12}$ = 2,1490
Ventromedial Caudate Putamen	56,0508± 2,6991	55,9778± 1,2748	51,5136± 6,7559	66,3797± 3,7985	63,8692± 5,8372	*p > 0.05*, $F_{4.13}$ = 1,6214
Vetrolateral Caudate Putamen	52,9175± 5,5881	57,5880± 4,4012	46,5360± 2,8874	65,4463± 2,4300	56,4579± 2,9212	*p > 0.05*, $F_{4.13}$ = 4,2348
Core of the Nucleus Accumbens	55,2001± 3,8810	62,2923± 1,7102	49,9710± 2,9031	64,9129± 5,1978	62,5464± 4,0793	*p > 0.05*, $F_{4.13}$ = 2,6253
Shell of the Nucleus Accumbens	49,4300± 6,1049	55,3990± 1,4661	48,0909± 3,9456	60,7672± 6,0609	51,4509± 5,0991	*p > 0.05*, $F_{4.13}$ = 1,1126

Table 5. mRNA levels of *Homer 1a* after acute ketamine treatment. Data are expressed as d.p.m. mean values±standard error means (S.E.M.) and listed by brain regions analyzed, along with the relative ANOVA (degrees of freedom and p values). Significant p values are expressed in bold. Post-hoc test: * significantly higher expression compared to VEH.

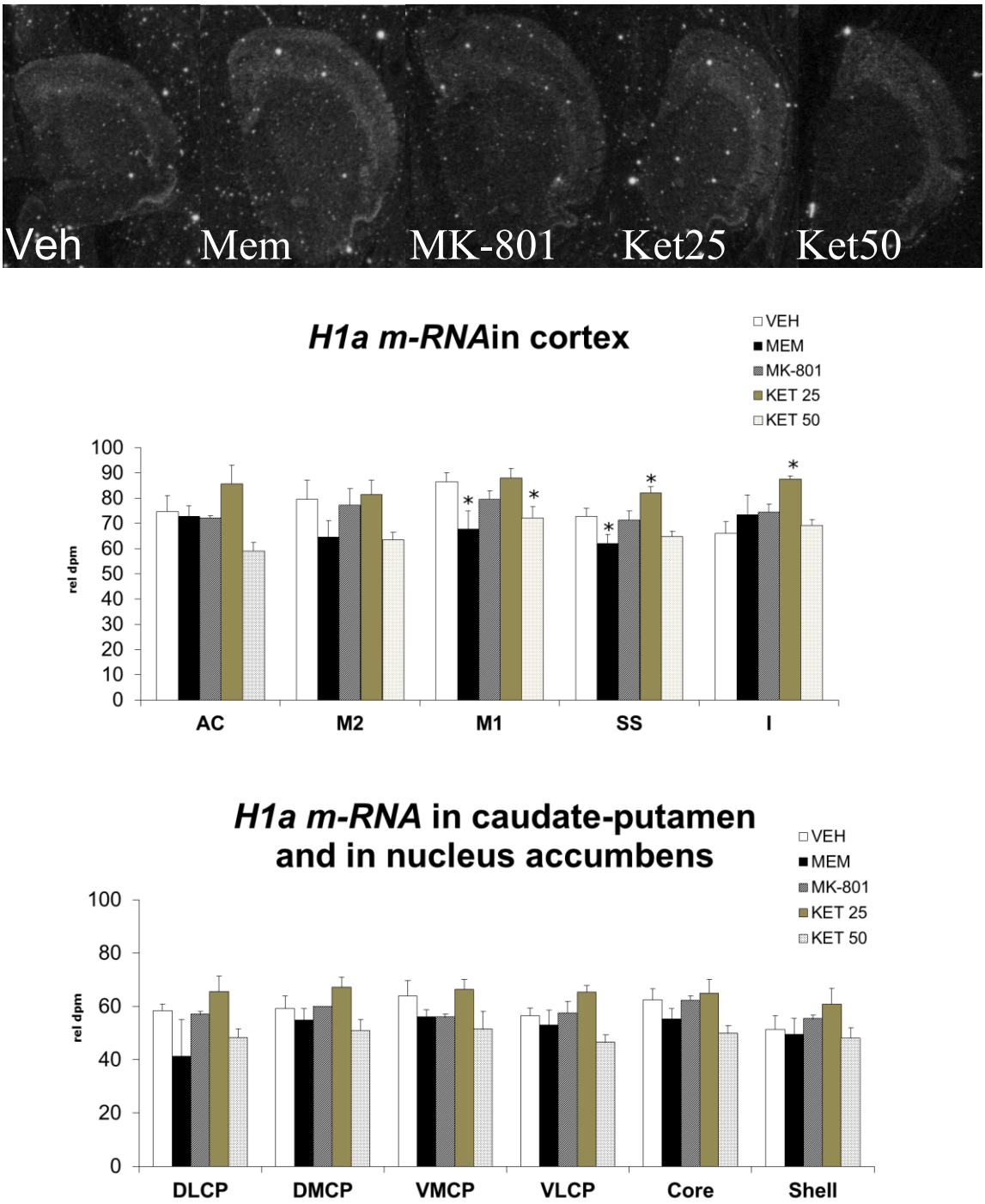
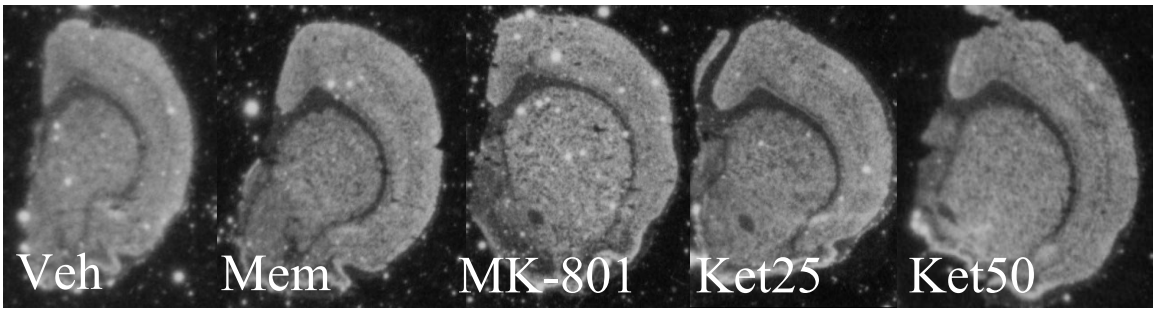
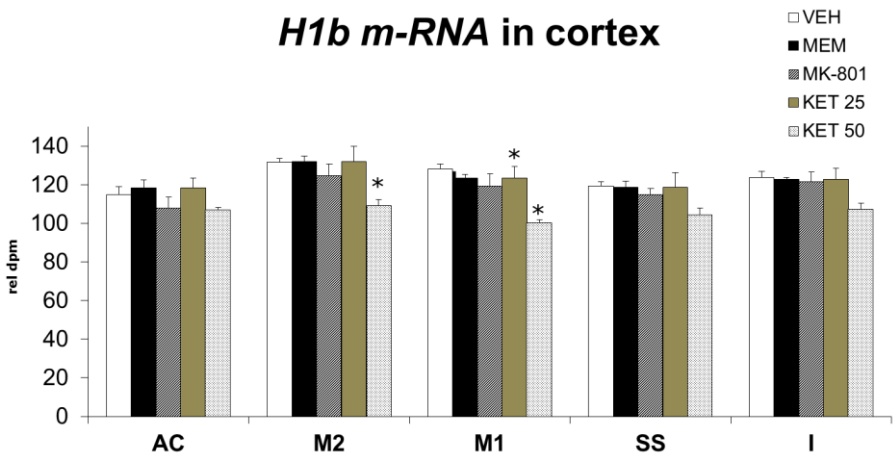


Figure 15. Autoradiographic image and graphics of *H1a* mRNA expression in Frontal Cortex, Striatum, Nucleus Accumbens expressed in relative dpm as mean ± S.E.M.; *ANOVA $p<0,05$ vs VEH.

Homer1b	**Memantine**	**MK-801**	**Ket 50**	**Ket 25**	**Veh**	**ANOVA (p, F)**
Frontal cortex						
Anterior Cingulate cortex	118,282± 4,0404	108,031± 5,7465	107,061± 1,2546	116,450± 5,2734	114,845± 4,1309	*p > 0.05,* $F_{4.15}$ = 1,3459
Medial Agranular Cortex	131,953± 2,7887	124,773± 5,9981	109,228± 3,2945*	119,030± 8,0328	131,765± 1,7012	***p = 0,0265,*** $F_{4.15}$ = 3,7385
Motor Cortex	123,335± 2,0169*	119,330± 6,4513	100,388± 1,5271*	109,222± 6,1989*	128,350± 2,2492	***p = 0,0023,*** $F_{4.13}$ = 6,9258
Somatosensory Cortex	118,668± 3,1867	115,048± 3,1126	104,508± 3,6391	108,251± 7,7392	119,459± 2,1100	*p > 0.05,* $F_{4.16}$ = 2,2249
Insular Cortex	122,942± 0,6542	121,780± 4,9081	107,304± 3,2584	119,187± 5,5163	123,777± 3,0918	*p > 0.05,* $F_{4.16}$ = 2,7019
Striatum						
Dorsomedial Caudate Putamen	100,578± 2,8868	106,435± 4,1457	99,281± 5,4546	97,534± 3,0980	108,007± 5,5382	*p > 0.05,* $F_{4.16}$ = 1,1012
Dorsolateral Caudate Putamen	110,250± 4,7136	111,475± 6,2786	97,478± 4,3687*	103,625± 4,1704*	124,283± 4,4151	***p =0,0249*** , $F_{4.16}$ = 3,7345
Ventromedial Caudate Putamen	111,618± 5,9833	115,529± 3,2496	113,317± 5,6941	107,563± 3,3377	118,657± 3,1007	*p > 0.05,* $F_{4.16}$ = 0,8826
Vetrolateral Caudate Putamen	118,659± 2,1440	124,462± 5,3995	110,342± 6,3813	112,226± 7,7323	123,203± 6,5800	*p > 0.05,* $F_{4.16}$ = 1,1617
Core of the Nucleus Accumbens	118,488± 3,4276	114,967± 3,2929	111,884± 5,1188	109,349± 3,7718	122,151± 3,6071	*p > 0.05,* $F_{4.16}$ = 1,6705
Shell of the Nucleus Accumbens	91,7777± 3,157	89,0715± 9,794	93,2689± 9,663	87,3565± 3,974	92,8403± 15,500	*p > 0.05,* $F_{4.16}$ = 0,0688

Table 6. mRNA levels of *Homer 1b* after acute ketamine treatment. Data are expressed as d.p.m. mean values±standard error means (S.E.M.) and listed by brain regions analyzed, along with the relative ANOVA (degrees of freedom and p values). Significant p values are expressed in bold. Post-hoc test: * significantly higher expression compared to VEH.





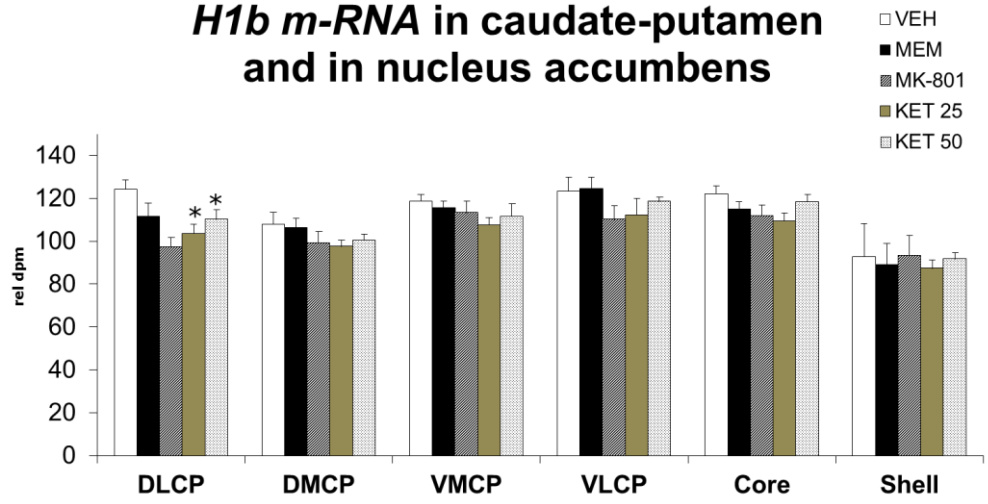
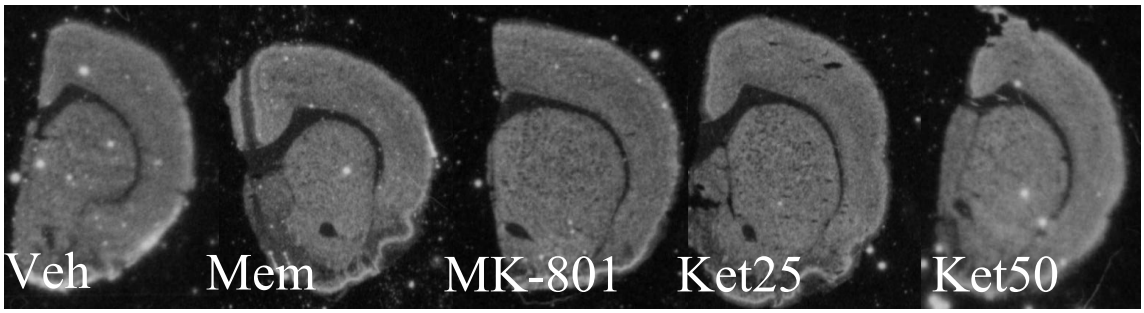


Figure 16. Autoradiographic image and graphics of *H1b* mRNA expression in Frontal Cortex, Striatum, Nucleus Accumbens expressed in relative dpm as mean ± S.E.M.; *ANOVA $p<0,05$ vs VEH.

PSD95	**Memantine**	**MK-801**	**Ket 50**	**Ket 25**	**Veh**	**ANOVA (p, F)**
Frontal cortex						
Anterior Cingulate cortex	194,858± 1,9492	174,968± 4,4275	193,219± 2,6314	185,995± 7,4056	192,808± 5,3335	*p > 0.05,* $F_{4.13}$ = 1,6903
Medial Agranular Cortex	190,192± 2,8010	183,609± 5,3810	187,529± 3,3665	180,433± 2,8656	192,790± 1,2344	*p > 0.05,* $F_{4.13}$ = 2,8369
Motor Cortex	179,688± 5,2426	176,066± 6,8308	174,621± 5,2798	175,396± 3,9123	183,763± 2,0368	*p > 0.05,* $F_{4.13}$ = 0,7411
Somatosensory Cortex	173,307± 3,1913	167,065± 3,0244	172,856± 3,5797	173,520± 2,1787	180,109± 1,5104	*p > 0.05,* $F_{4.14}$ = 3,2306
Insular Cortex	191,434± 2,0232	184,708± 3,5258	191,297± 0,2318	183,562± 4,2627	186,233± 4,0871	***p = 0,4740,*** $F_{4.14}$ = 0,9311
Striatum						
Dorsomedial Caudate Putamen	177,538± 3,0961	170,670± 2,4602*	*175,9053*± 2,9123*	*176,473*± 2,1923*	185,297± 3,2757	***p =0,0270*** , $F_{4.14}$ = 3,8006
Dorsolateral Caudate Putamen	182,199± 3,3377	166,778± 1,7327*	174,647± 3,4661	174,046± 3,6187	181,402± 1,5383	***p =0,0158*** , $F_{4.14}$ = 4,4457
Ventromedial Caudate Putamen	186,929± 4,0533	168,983± 2,8131*	180,164± 1,0510	179,676± 4,2777	183,647± 3,6382	***p =0,0431,*** $F_{4.14}$ = 3,2747
Vetrolateral Caudate Putamen	188,993± 3,6656	178,758± 3,9901	183,346± 3,8891	186,202± 4,8988	181,942± 4,5282	*p > 0.05,* $F_{4.14}$ = 0,7261
Core of the Nucleus Accumbens	192,150± 0,6204	180,430± 0,6946	188,528± 2,0450	190,689± 4,5539	190,039± 2,9205	*p > 0.05,* $F_{4.14}$ = 2,1179
Shell of the Nucleus Accumbens	179,294± 5,6548	180,132± 3,3519	178,496± 6,0050	175,140± 5,6075	191,149± 1,6767	*p > 0.05,* $F_{4.13}$ = 1,3638

Table 7. mRNA levels of *PSD95* after acute ketamine treatment. Data are expressed as d.p.m. mean values±standard error means (S.E.M.) and listed by brain regions analyzed, along with the relative ANOVA (degrees of freedom and p values). Significant p values are expressed in bold. Post-hoc test: * significantly higher expression compared to VEH.



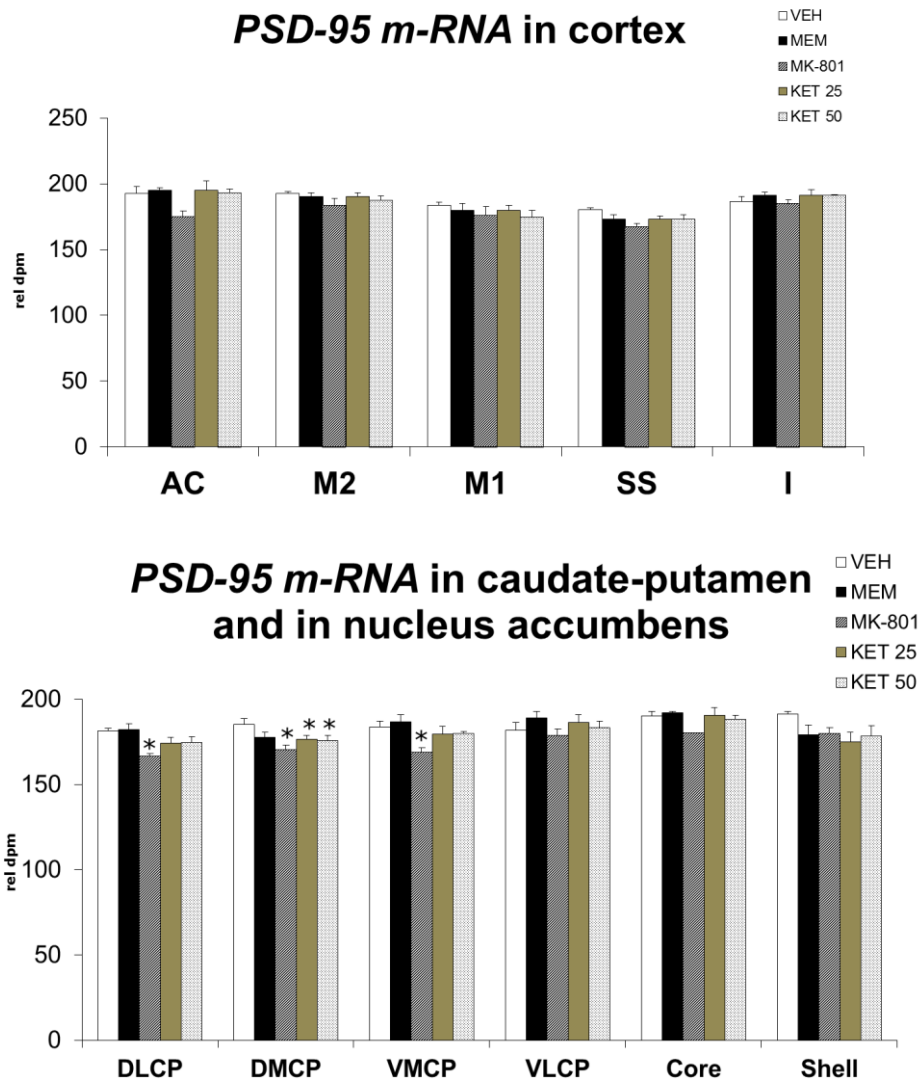


Figure 17. Autoradiographic image and graphics of PSD95 mRNA expression in Frontal Cortex, Striatum, Nucleus Accumbens expressed in relative dpm as mean ± S.E.M.; *ANOVA $p<0,05$ vs VEH.

Chapter 6.

Discussion.

In the first paradigm studied herein, we aimed to gain knowledge into the putative connection between psychosis and glucose metabolism. Therefore, we chose to treat rats with sub-anesthetic doses of ketamine, a compound assumed to provide a pharmacological animal model of psychosis by its action as a non-competitive antagonist of glutamatergic NMDA-Rs (Bubenikova-Valesova et al. 2008). Beyond its action on neurotransmitter systems, ketamine is believed to induce psychosis-resembling behaviors in rodents by triggering neurotoxic damage and apoptosis in selected brain areas (Green and Cote 2009).

In this model, we chose to study the expression of *Hk1* and *GLUT3* genes, which code for the brain-specific isoforms of these molecules and may be considered candidate genes for both psychosis and glucose metabolism dysfunctions (Prabakaran et al. 2004; McDermott and de Silva 2005; Robey and Hay 2006; Martins-de-Souza et al. 2009; Martins-De-Souza et al. 2010).

We have found that ketamine dose-dependently affects the expression of both *Hk1* and *GLUT3* in a region-specific fashion and may change the topographic distribution of gene expression in the regions of interest when compared to basal distribution. The neurotoxic dose of ketamine (50 mg/kg) has been observed to increase *Hk1* gene expression in several cortical and subcortical areas, while the non-neurotoxic dose (12 mg/kg) did not affect increased *Hk1* expression in the majority of regions studied auditory cortex only. On the contrary, *GLUT3* expression was decreased by the 50-mg/kg dose of ketamine has been shown to decrease *GLUT3* expression in the retrosplenial cortex and in striatum, lateral septum, and frontal cortex subcortical areas

and increased by 12 mg/kg ketamine in the frontal cortex and the striatum (although in this latter region compared to KET50 only).

These findings appear to confirm the view that Hk1 and GLUT3 may be implicated in ketamine-induced metabolic dysfunctions and may play a role in molecular mechanisms of psychosis. As ketamine acts preferentially as an antagonist of NMDA-Rs, it could be hypothesized that glutamate perturbation may secondarily affect glucose metabolism, thereby stimulating *Hk1* expression as a feedback compensatory mechanism. Consistent with this view, a reduction of glycolysis has been found in cortical areas of rats treated with MK-801, another NMDA-R antagonist believed to provide a pharmacological model of psychosis (Eyjolfsson et al. 2011). Moreover, hypoglycemia has been associated with enhanced cortical expression of genes coding for NMDAR1, NMDA2B and mGlu5 receptors (Joseph et al. 2008). Glutamatergic synapses have recently received great attention as putative sites of lesions in psychosis pathophysiology (Olney et al. 1999). In our previous studies, we have observed that the expression of the immediate early-gene *Homer1a* is modulated by antipsychotics (Iasevoli et al. 2010) and by a dopamine D2R selective antagonist (Iasevoli et al. 2009) as well as by ketamine (Iasevoli et al. 2007). These observations lend further support to the view that molecules involved in glutamatergic neurotransmission within glutamatergic synapses may be implicated in the pathophysiology of psychosis or in the molecular mechanisms of action of anti-psychotic drugs. Hk1 may thus represent a novel molecular target to study glutamatergic dysfunctions in psychosis.

Increased *Hk1* expression may also represent a mechanism to escape neurotoxic damage and apoptosis induced by ketamine. Ketamine is known to induce apoptosis in neuronal cell lines via the mitochondrial pathway (Braun et al. 2010) and sub-anesthetic ketamine has been found to trigger mitochondrial dysfunctions in prefrontal cortex, striatum and

hippocampus of rats (de Oliveira et al. 2011). Intriguingly, Hk1 competes with the pro-apoptotic Bax/Bak factors to interact with mitochondria (Pastorino et al. 2002). Detachment of Hk1 from mitochondria may facilitate binding to Bax/Bak and may dys-regulate mitochondrial VDAC functions (Gottlieb et al. 2003), both phenomena promoting apoptosis. Induction of apoptosis has been associated with decreased Hk1 association with mitochondria (Gottlieb 2001). Thus, increase of *Hk1* gene expression increase by ketamine may imply a compensatory mechanism for triggered by activation of apoptotic pathways. The preferential increase by the neurotoxic dose of ketamine appears to support this suggestion. Notably, *Hk1* expression has been found reduced in schizophrenia brain tissue (Prabakaran et al. 2004; Martins-de-Souza et al. 2009). It could be hypothesized that reduced or insufficient *Hk1* expression may fail prevention of apoptosis and thus predispose to neurotoxic damage, which has been accounted for schizophrenia pathophysiology.

Although ketamine has been shown to increase *Hk1* gene expression according to a region-specific distribution, the pattern of *Hk1* gene expression is strikingly divergent from that of other genes, e.g. the immediate-early gene *c-fos* (Imre et al. 2006). This peculiar pattern may depend on superior sensitivity of some brain areas compared to others in terms of ketamine-mediated dysfunctions, at least in glucose metabolism. Indeed, auditory and retrosplenial cortex appeared to be a sensible brain areas, as Hk1 expression has been found increased by both drug doses and gene expression distribution was significantly modified compared to basal distribution by ketamine administration.

Auditory, visual and retrosplenial cortices, as well as several aspects of striatum belong to a complex neuronal network controlling and influenced by emotive and cognitive behavior (Lang et al. 1998; Maddock 1999; Radwanska et al. 2010; Simpson et al.

2010; Plichta et al. 2011). *Hk1* expression in these areas was more consistently affected by ketamine compared to sensor-motor regions, e.g.: subregions of the frontal cortex. Disruption of working memory, cognitive tasks, as well as induction of emotional blunting have been described in rats exposed to ketamine (Imre et al. 2006; Pietersen et al. 2007) and ketamine administration has been proposed as an effective model of cognitive deficits and negative symptoms of schizophrenia (Krystal et al. 1994; Neill et al. 2010). The results of this study may suggest that ketamine prominently induces neurotoxic damage in brain regions implicated in the regulation of emotive and cognitive behavior. More studies are needed to test this hypothesis.

It has been observed that GLUT3 surface expression and glucose import is enhanced by synaptic stimulation in cultured cortical and hippocampal neurons. This effect is counteracted by inhibition of NMDA-Rs, thus demonstrating that activation of NMDA-Rs increases GLUT3 surface expression and activity (Ferreira et al. 2011). The lack or the reduction of *GLUT3* gene increase by ketamine described herein seems to be consistent with these observations. Taken together, these results let hypothesize that GLUT3 may tune glucose uptake into neurons in relation to the extent of NMDA-R-mediated activation.

In the second paradigm of this study, we explored putative changes in genes relevant for dopamine signaling, namely the genes coding for dopamine D1R and D2R and for DAT, after either acute or subchronic exposure to systemic ketamine. The rationale for this study stems from the observations that ketamine, and other NMDA-R antagonists, may induce a psychotic state in humans and schizophrenia-resembling behaviors in rodents that are reverted by anti-dopaminergic agents, as antipsychotics (Lipska and Weinberger 2000). Indeed, NMDA-R antagonists as ketamine are known to affect dopamine release and turnover (Javitt 2010) and possibly dopamine signaling.

Moreover, it has been suggested that acute NMDA-R blockade may resemble acute psychotic symptoms while subchronic antagonism at NMDA-Rs may best model long-lasting cognitive and molecular changes occurring in psychosis (Krystal et al. 1994; Neill et al. 2010). Thereby, we wanted to investigate whether putative gene changes may be different in acute *vs*. subchronic ketamine administration.

Dopamine *D1R* expression was decreased by acute ketamine in the lateral caudate-putamen and in the core of the accumbens while expression in the cortex was not significantly changed. Dopamine *D2R* expression was not affected by acute ketamine. After subchronic exposure to ketamine, however, *D1R* expression was not affected in any of the region assessed, while *D2R* expression was increased in the ventrotegmental area and *DAT* expression was increased in the ventrotegmental area and the substantia nigra pars compacta. Both the temporal and topographic profile of gene expression changes are suggestive of feedback adaptations in dopamine system occurring as a consequence of ketamine-induced dopamine perturbation and aiming at preserving homeostasis.

The results described above suggest that acute response to ketamine takes place with regard to dopamine gene expression changes at postsynaptic sites, while presynaptic feedback mechanisms could be activated after prolonged exposure to ketamine. Moreover, the results seem also to suggest that subcortical areas may be more sensitive than cortical areas to the effects of ketamine-mediated dopaminergic dysfunction, at least in terms of adaptive changes of gene expression.

Acute ketamine has been described to induce dopamine release in both cortex and striatum (Verma and Moghaddam 1996). Decrease of *D1R* expression in striatal subregions may be due to a mechanism of regulation putatively induced by the dopaminergic subcortical overstimulation promoted by acute ketamine administration.

The lack of relevant *D1R* expression changes in cortex may imply that mechanisms different from changes in gene expression may take place in this region to compensate hyperdopaminergia. Moreover, it may be not excluded that increased activity of D1 receptors may represent a beneficial mechanism to counteract ketamine-induced NMDA-R hypofunction. Indeed, it has been demonstrated that NMDA and D1 receptors may interact physically and functionally (Kruse et al. 2009). Indeed, there is some evidence that increasing D1R activity in the prefrontal cortex enhances NMDA-R function (Li et al. 2010), while in hippocampal neurons activation of D1R has been shown to upregulate NMDA-R-mediated LTP (Nai et al. 2010).

In the subchronic paradigm, the absence of significant changes in the expression of post-synaptic D1R is consistent with the view that prolonged exposure to ketamine and prolonged hyperdopaminergia may be best counteracted by presynaptic rather than postsynaptic mechanisms, once these have failed to revert hyperdopaminergia. Indeed, the increase of *DAT* and *D2R* expression in the subchronic paradigm is consistent with this view.

These data are in agreement with previous studies. It has been suggested that chronic ketamine may induce functional hypersensitivity of dopamine-releasing mechanisms, probably through increased sensitivity of DAT located on pre-synaptic dopaminergic neurons. These presynaptic mechanisms, at least partially and at level of transcription, could underlie the facilitation of stimulus-related dopamine release induced by ketamine.

Considering previous evidence of direct modulation of DAT function by D2R autoreceptor, our results could fit with the above-mentioned hypothesis. Subchronic administration of ketamine may augment the effects of psychostimulants in drug-abuse

situations. Similar pre-synaptic hypersensitivity could contribute to the development of "endogenous sensitization" in the pathophysiology of psychosis.

In humans, ketamine has been shown to increase dopamine release in striatum and to sensitize dopamine system, enhancing amphetamine-mediated dopamine release (Kegeles et al. 2000). Albeit acting on glutamate transmission, ketamine induces profound changes in dopamine signalling, consistent with its potential to elicit psychotic symptoms in healthy humans and to exacerbate psychosis in stable schizophrenics. Nonetheless, molecular changes in dopamine system after acute or chronic ketamine administration are poorly understood, also because of ethical issues in studying chronic ketamine in human experiments. The depiction of molecular changes in a condition of ketamine-induced hyperdopaminergia may have great relevance as they may resemble those putatively occurring in schizophrenia pathophysiology. The picture emerging from the experiments carried out in this study suggests that striatum and midbrain are more interested than cortical regions, at least in terms of gene expression, in a condition of ketamine-mediated dopamine perturbation and that postsynaptic mechanisms may represent a first line adaptations that may be replaced by presynaptic mechanisms once failed to revert hyperdopaminergia.

Administration of a set of NMDA-R antagonists has been found to trigger different changes in the expression of genes involved in glutamate signaling, neuronal functioning, and marking neuronal activity status (i.e.: *Homer 1a, Arc, Homer 1b, PSD95* and *c-fos*). The differences in gene expression observed in this study are consistent with the different clinical and preclinical profile of these compounds, albeit their apparently similar pharmacological mechanisms. It appears that the different

NMDA-R antagonists used may activate divergent post-receptor pathways of signal transduction and neuronal activation and may act on different brain regions.

Ketamine and MK-801 induce a psychotic state in humans and schizophrenia-resembling behaviors in animals and are widely used to provide animal models of the disease. On the other hand, the NMDA-R antagonist memantine is currently in use as a therapeutic agent in cognitive failure and has been proposed in the treatment of negative and cognitive symptoms of schizophrenia, as add-on to traditional antipsychotics. According to the prevalent pro-cognitive action of the compound, memantine affects gene expression exclusively in cortical areas. Non-competitive NMDA-R antagonists, above all MK-801 and the neurotoxic subanaesthetic dose of ketamine, affect the expression of both inducible and constitutive genes in both cortical and subcortical areas, a feature that is consistent with the strong locomotor action by these compounds (de Bartolomeis and Iasevoli 2003; Iasevoli et al. 2007). *Arc* and *c-fos* expression are increased by memantine in the cortex, presumably as a consequence of neuronal activation in this area. *Arc* expression is increased in ventral striatum by ketamine 50mg/kg and MK-801. Arc protein interacts with proteins involved in clathrin-mediated endocytosis and facilitates the removal of AMPA-R from the membrane (Chowdhury et al. 2006; Bloomer et al. 2007). These plastic changes could modulate synaptic activation and prevent hyper-activation/neurotoxicity of glutamatergic neurons, important for learning and memory (McIntyre et al. 2005). It may be expected that, beyond marking neuronal activation, the relevant *Arc* induction by ketamine and MK-801 may also have a mechanistic value, in order to prevent hyper-activation of postsynaptic receptors.

Expression of the inducible gene *Homer1a* is decreased rather than induced by memantine. In previous studies, expression of *Homer1a* has been found increased by

the non-competitive antagonist ketamine and by antagonism at D2Rs. *Homer1a* expression has been conceptualized as being influenced by glutamate action onto non-NMDA-Rs and by dopamine action onto D1Rs. Although blocking NMDA-Rs, memantine action may be best simplified as consisting in a partial agonism at NMDA-Rs (Johnson and Kotermanski 2006), thus suggesting a state of functional agonism/antagonism depending on endogenous glutamate activity. It is expected that memantine may not cause a condition of NMDA-R hypofunction, as that described for ketamine, and thus it may not induce paradoxical hyperglutamatergia onto non-NMDA-Rs. Moreover, memantine has been described to exert agonist action at D2Rs. These features may explain the lack of *Homer 1a* induction or its reduction by memantine.

Conclusions:

In the studies described here, we have explored the effect of ketamine, as a NMDA-R antagonist providing a powerful animal model of psychosis, on key genes of dopamine and glutamate postsynaptic neurotransmission, neuronal activation, and glucose metabolism in neurons. Our aim was to investigate the molecular changes putatively occurring in multiple biological systems in an animal model that has been widely used to resemble psychotic-like behaviors in preclinical paradigms and that is considered to have high heuristic, construct, and predictive validity (Lipska and Weinberger 2000).

Overall, the results show that ketamine administration may be associated with adaptive changes in glucose metabolism and dopamine signaling. Glucose metabolism may be impaired by ketamine, causing an increase in the expression of *Hk1* gene (coding for the enzyme catalyzing glycolysis), and a decrease in the expression of the *GLUT3* (coding for the main membrane transporter involved in glucose intake within neurons). Hexokinase has also been shown to exert an anti-apoptotic and protective effect in neurons. Ketamine is known to cause neuron damage. Hexokinase transcription increase after ketamine administration may thus represent a feedback mechanism to prevent neurotoxicity induced by this compound.

Acute ketamine has been described to increase dopamine release in striatum (Iasevoli et al. 2007) while subchronic ketamine appears to induce dopamine sensitization (Becker et al. 2003). According to these data, in our study we have found that acute ketamine reduces *D1R* expression. D1R are the main postsynaptic excitatory dopamine receptors. Increased dopamine release induces a hyperstimulation of D1R and thus a condition of postsynaptic hyperdopaminergia. Reduction of *D1R* expression may represent a mechanism to counteract this acute hyperdopaminergia. Subchronic ketamine increases dopamine *D2R* and *DAT* expression, which may represent a mechanism to reduce

dopamine turnover and synthesis and ultimately dopamine release; and to reduce dopamine levels in the synaptic cleft. Thus, also these molecular changes may reflect a feedback mechanism to avoid hyperdopaminergia.

However, the molecular changes induced by ketamine, and by the similar compound MK-801, are straightly divergent from those induced by memantine, another NMDA-R antagonist that has been observed to have pro-cognitive effects and that has been proposed as add-on agent to antipsychotics.

Subanaesthetic doses of ketamine are known to induce a complex psychotic state in humans. Acute ketamine has been demonstrated to induce a worsening of BPRS scores in schizophrenic individuals (Lahti et al. 2001). Subchronic ketamine administration has been described to induce both positive and negative symptoms of psychosis, perceptual alterations and an impairment in several cognitive tasks (Krystal et al. 1994). Moreover, ketamine has been shown to affect metabolic parameters in humans. Regional cerebral blood flow has been found altered in limbic regions of schizophrenic individuals undergoing ketamine exposure (Lahti et al. 1995). Brain glucose metabolic rate in CNS has been found affected in several cortical regions and associated with psychopathology in healthy volunteers administered with ketamine (Vollenweider et al. 1997).

Nonetheless, little is known about molecular changes occurring as a consequence of ketamine administration and that may resemble the molecular perturbation taking place in psychosis pathophysiology. The studies carried out and described herein allows to get insights into these issues and to provide spatial and temporal profiles of molecular changes elicited by ketamine administration to rats. It appears that ketamine may trigger a complex network of molecular adaptations, involving the glutamate and the dopamine systems and glucose metabolism. However, the topography of molecular changes seems to be peculiar for each system. Acute ketamine-mediated dopamine perturbation has

been described to trigger gene changes in the striatum only, while chronic perturbation by ketamine appears to induce changes in the midbrain but not in the striatum. No significant changes in dopamine genes have been recognized in the cortex. This specific pattern is consistent with the view that acute ketamine may trigger postsynaptic adaptations, while chronic ketamine may induce presynaptic feedback mechanisms to occur. Subcortical hyperdopaminergia has been related to positive symptoms of psychosis, that have been described with ketamine (Lahti et al. 1995). In opposition to what observed for dopamine receptors and dopamine transporter gene expression, the transcription of genes implicated in neuronal activation and glutamate signaling is prominently affected in cortical regions in the present study, although a previous study has also described a modulation of glutamatergic genes in ventral striatum by ketamine (Iasevoli et al. 2007). This pattern is consistent with the view that both glutamate and cortical impairment may be implicated in cognitive failure in psychosis that has also been described with ketamine (Krystal et al. 1994). Moreover, ketamine administration to rodents has been reported to induce several neuropathological lesions in cortical regions (Carlsson and Svensson 1990; Tiedtke et al. 1990; Riederer et al. 1991; Schmidt et al. 1991). Toxicity on cortical neurons has been hypothesized as the ultimate and diseases-inducing step of the NMDA-R hypofunction hypothesis of psychosis pathophysiology, that has been proposed also taking in account human and animal studies on behavioral, biochemical and histopathological correlates of ketamine administration (Allen and Iversen 1990; Olney et al. 1991; Sharp et al. 1994). In agreement with these observations and consistent with the data deriving from the study of glutamatergic genes, we have described that ketamine may strongly affect the expression of two key genes (i.e.: Hk1 and GLUT 3) of brain glucose metabolism in several cortical regions. These observations are consistent with early human studies on

alterations of cerebral metabolic rates by the compound (Vollenweider et al. 1997). Moreover, Hk1 has anti-apoptotic activity. The strong increase of *Hk1* expression by ketamine in the cortex may also imply that ketamine exposure activates anti-apoptotic mechanisms to prevent the toxic neuronal damage that has been described with this agent. Intriguingly, memantine, a partial agonist/antagonist at NMDA-Rs, has anti-apoptotic and pro-cognitive potential. Albeit acting as a NMDA-R antagonist in some cases, in our experiments memantine induces different and somewhat opposite molecular changes when compared to the fully NMDA-R antagonists ketamine and MK-801. Memantine-induced changes are prevalent in cortical regions as in the case of ketamine, suggesting that the fine-modulation of NMDA-R activity, even in the same brain regions, may activate divergent intracellular pathways and may give raise to divergent biological and, perhaps, behavioral consequences, possibly explaining the divergent clinical outcomes of these compounds.

The overall conclusion that stems from the different paradigms investigated in this study and based on subanaesthetic ketamine administration model of psychosis in rats is that multiple changes in gene expression occur after NMDA-R-blockade in cortical and subcortical regions and affect the transcription of genes involved in glucose metabolism and dopamine-glutamate interaction. These results may represent an initial insight into the molecular basis of metabolic and neurotransmitter changes that have been reported in vivo in schizophrenics patients as well as in normal subjects after ketamine administration. More studies are warranted to explore the relationship between the perturbation of dopamine–glutamate interaction and glucose metabolism in the brain using ketamine and other NMDA–R non competitive antagonists as animal models of psychosis-like disorder.

References.

Abi-Dargham, A., R. Gil, et al. (1998). "Increased striatal dopamine transmission in schizophrenia: confirmation in a second cohort." Am J Psychiatry **155**(6): 761-767.

Abi-Dargham, A., O. Mawlawi, et al. (2002). "Prefrontal dopamine D1 receptors and working memory in schizophrenia." J Neurosci **22**(9): 3708-3719.

Adams, B. and B. Moghaddam (1998). "Corticolimbic dopamine neurotransmission is temporally dissociated from the cognitive and locomotor effects of phencyclidine." J Neurosci **18**(14): 5545-5554.

Adler, C. M., A. K. Malhotra, et al. (1999). "Comparison of ketamine-induced thought disorder in healthy volunteers and thought disorder in schizophrenia." Am J Psychiatry **156**(10): 1646-1649.

Afshar, H., H. Roohafza, et al. (2009). "Topiramate add-on treatment in schizophrenia: a randomised, double-blind, placebo-controlled clinical trial." J Psychopharmacol **23**(2): 157-162.

Allen, H. L. and L. L. Iversen (1990). "Phencyclidine, dizocilpine, and cerebrocortical neurons." Science **247**(4939): 221.

Allen, R. M. and S. J. Young (1978). "Phencyclidine-induced psychosis." Am J Psychiatry **135**(9): 1081-1084.

Ambesi-Impiombato, A., G. D'Urso, et al. (2003). "Method for quantitative in situ hybridization histochemistry and image analysis applied for Homer1a gene expression in rat brain." Brain Res Brain Res Protoc **11**(3): 189-196.

Ambesi-Impiombato, A., F. Panariello, et al. (2007). "Differential expression of Homer 1 gene by acute and chronic administration of antipsychotics and dopamine transporter inhibitors in the rat forebrain." Synapse **61**(6): 429-439.

Andreassen, O. A., T. O. Aamo, et al. (1996). "Inhibition by memantine of the development of persistent oral dyskinesias induced by long-term haloperidol treatment of rats." Br J Pharmacol **119**(4): 751-757.

Awad, H., G. W. Hubert, et al. (2000). "Activation of metabotropic glutamate receptor 5 has direct excitatory effects and potentiates NMDA receptor currents in neurons of the subthalamic nucleus." J Neurosci **20**(21): 7871-7879.

Balla, A., R. Koneru, et al. (2001). "Continuous phencyclidine treatment induces schizophrenia-like hyperreactivity of striatal dopamine release." Neuropsychopharmacology **25**(2): 157-164.

Beaulieu, J. M., T. D. Sotnikova, et al. (2005). "An Akt/beta-arrestin 2/PP2A signaling complex mediates dopaminergic neurotransmission and behavior." Cell **122**(2): 261-273.

Beaulieu, J. M., T. D. Sotnikova, et al. (2004). "Lithium antagonizes dopamine-dependent behaviors mediated by an AKT/glycogen synthase kinase 3 signaling cascade." Proc Natl Acad Sci U S A **101**(14): 5099-5104.

Becker, A., B. Peters, et al. (2003). "Ketamine-induced changes in rat behaviour: A possible animal model of schizophrenia." Prog Neuropsychopharmacol Biol Psychiatry **27**(4): 687-700.

Bellivier, F. (2005). "Schizophrenia, antipsychotics and diabetes: Genetic aspects." Eur Psychiatry **20 Suppl 4**: S335-339.

Block, F. and M. Schwarz (1996). "Memantine reduces functional and morphological consequences induced by global ischemia in rats." Neurosci Lett **208**(1): 41-44.

Bloomer, W. A., H. M. VanDongen, et al. (2007). "Activity-regulated cytoskeleton-associated protein Arc/Arg3.1 binds to spectrin and associates with nuclear promyelocytic leukemia (PML) bodies." Brain Res **1153**: 20-33.

Bottai, D., J. F. Guzowski, et al. (2002). "Synaptic activity-induced conversion of intronic to exonic sequence in Homer 1 immediate early gene expression." J Neurosci **22**(1): 167-175.

Braun, S., N. Gaza, et al. (2010). "Ketamine induces apoptosis via the mitochondrial pathway in human lymphocytes and neuronal cells." Br J Anaesth **105**(3): 347-354.

Breier, A., T. P. Su, et al. (1997). "Schizophrenia is associated with elevated amphetamine-induced synaptic dopamine concentrations: Evidence from a novel positron emission tomography method." Proceedings of the National Academy of Sciences of the United States of America **94**(6): 2569-2574.

Bubenikova-Valesova, V., J. Horacek, et al. (2008). "Models of schizophrenia in humans and animals based on inhibition of NMDA receptors." Neurosci Biobehav Rev **32**(5): 1014-1023.

Cardenas, M. L., A. Cornish-Bowden, et al. (1998). "Evolution and regulatory role of the hexokinases." Biochim Biophys Acta **1401**(3): 242-264.

Carlsson, A. (1988). "The current status of the dopamine hypothesis of schizophrenia." Neuropsychopharmacology **1**(3): 179-186.

Carlsson, A. and M. Lindqvist (1963). "Effect of Chlorpromazine or Haloperidol on Formation of 3methoxytyramine and Normetanephrine in Mouse Brain." Acta Pharmacol Toxicol (Copenh) **20**: 140-144.

Carlsson, M. and A. Svensson (1990). "The non-competitive NMDA antagonists MK-801 and PCP, as well as the competitive NMDA antagonist SDZ EAA494 (D-CPPene), interact synergistically with clonidine to promote locomotion in monoamine-depleted mice." Life Sci **47**(19): 1729-1736.

Carr, D. B. and S. R. Sesack (2000). "Projections from the rat prefrontal cortex to the ventral tegmental area: target specificity in the synaptic associations with mesoaccumbens and mesocortical neurons." J Neurosci **20**(10): 3864-3873.

Caumont, A. S., J. N. Octave, et al. (2006). "Amantadine and memantine induce the expression of the glial cell line-derived neurotrophic factor in C6 glioma cells." Neurosci Lett **394**(3): 196-201.

Chen, H. S., J. W. Pellegrini, et al. (1992). "Open-channel block of N-methyl-D-aspartate (NMDA) responses by memantine: therapeutic advantage against NMDA receptor-mediated neurotoxicity." J Neurosci **12**(11): 4427-4436.

Chen, P. C., C. L. Lao, et al. (2007). "Dual alteration of limbic dopamine D1 receptor-mediated signalling and the Akt/GSK3 pathway in dopamine D3 receptor mutants during the development of methamphetamine sensitization." J Neurochem **100**(1): 225-241.

Cheng, M. C., C. L. Lu, et al. (2010). "Genetic and functional analysis of the DLG4 gene encoding the post-synaptic density protein 95 in schizophrenia." PLoS One **5**(12): e15107.

Cho, K. O., C. A. Hunt, et al. (1992). "The rat brain postsynaptic density fraction contains a homolog of the Drosophila discs-large tumor suppressor protein." Neuron **9**(5): 929-942.

Chowdhury, S., J. D. Shepherd, et al. (2006). "Arc/Arg3.1 interacts with the endocytic machinery to regulate AMPA receptor trafficking." Neuron **52**(3): 445-459.

Coerver, K. A., S. M. Gray, et al. (1998). "Developmental expression of hexokinase 1 and 3 in rats." Histochem Cell Biol **109**(1): 75-86.

Creese, I., D. R. Burt, et al. (1976). "Dopamine receptor binding predicts clinical and pharmacological potencies of antischizophrenic drugs." Science **192**(4238): 481-483.

Davidson, L. L. and R. W. Heinrichs (2003). "Quantification of frontal and temporal lobe brain-imaging findings in schizophrenia: a meta-analysis." Psychiatry Res **122**(2): 69-87.

Davis, K. L., R. S. Kahn, et al. (1991). "Dopamine in schizophrenia: a review and reconceptualization." Am J Psychiatry **148**(11): 1474-1486.

Day, H. E., E. M. Kryskow, et al. (2008). "Conditioned fear inhibits c-fos mRNA expression in the central extended amygdala." Brain Res **1229**: 137-146.

de Bartolomeis, A., L. Aloj, et al. (2002). "Acute administration of antipsychotics modulates Homer striatal gene expression differentially." Brain Res Mol Brain Res **98**(1-2): 124-129.

de Bartolomeis, A., G. Fiore, et al. (2005). "Dopamine-glutamate interaction and antipsychotics mechanism of action: implication for new pharmacological strategies in psychosis." Curr Pharm Des **11**(27): 3561-3594.

de Bartolomeis, A. and F. Iasevoli (2003). "The Homer family and the signal transduction system at glutamatergic postsynaptic density: potential role in behavior and pharmacotherapy." Psychopharmacol Bull **37**(3): 51-83.

de Oliveira, M. R., R. F. da Rocha, et al. (2011). "Total and mitochondrial nitrosative stress, decreased brain-derived neurotrophic factor (BDNF) levels and glutamate uptake, and evidence of endoplasmic reticulum stress in the hippocampus of vitamin A-treated rats." Neurochem Res **36**(3): 506-517.

Delay, J., P. Deniker, et al. (1952). "[Therapeutic use in psychiatry of phenothiazine of central elective action (4560 RP)]." Ann Med Psychol (Paris) **110**(2:1): 112-117.

Dell'aversano, C., C. Tomasetti, et al. (2009). "Antipsychotic and antidepressant co-treatment: effects on transcripts of inducible postsynaptic density genes possibly implicated in behavioural disorders." Brain Res Bull **79**(2): 123-129.

Dursun, S. M. and S. Devarajan (2001). "Accelerated weight loss after treating refractory depression with fluoxetine plus topiramate: possible mechanisms of action?" Can J Psychiatry **46**(3): 287-288.

Dwyer, D. S., H. B. Pinkofsky, et al. (1999). "Antipsychotic drugs affect glucose uptake and the expression of glucose transporters in PC12 cells." Prog Neuropsychopharmacol Biol Psychiatry **23**(1): 69-80.

Ehrlich, M., E. Knolle, et al. (1999). "Memantine for prevention of spinal cord injury in a rabbit model." J Thorac Cardiovasc Surg **117**(2): 285-291.

Eisenberg, E., B. P. Vos, et al. (1993). "The NMDA antagonist Memantine blocks pain behavior in a rat model of formalin-induced facial pain." Pain **54**(3): 301-307.

Emamian, E. S., D. Hall, et al. (2004). "Convergent evidence for impaired AKT1-GSK3beta signaling in schizophrenia." Nat Genet **36**(2): 131-137.

Erritzoe, D., P. Talbot, et al. (2003). "Positron emission tomography and single photon emission CT molecular imaging in schizophrenia." Neuroimaging Clin N Am **13**(4): 817-832.

Eyjolfsson, E. M., L. H. Nilsen, et al. (2011). "Altered 13C glucose metabolism in the cortico-striato-thalamo-cortical loop in the MK-801 rat model of schizophrenia." J Cereb Blood Flow Metab **31**(3): 976-985.

Farber, N. B. (2003). "The NMDA receptor hypofunction model of psychosis." Ann N Y Acad Sci **1003**: 119-130.

Farber, N. B., S. H. Kim, et al. (2002). "Receptor mechanisms and circuitry underlying NMDA antagonist neurotoxicity." Mol Psychiatry **7**(1): 32-43.

Ferreira, J. M., A. L. Burnett, et al. (2011). "Activity-dependent regulation of surface glucose transporter-3." J Neurosci **31**(6): 1991-1999.
Foa, L. and R. Gasperini (2009). "Developmental roles for Homer: more than just a pretty scaffold." J Neurochem **108**(1): 1-10.
Gardoni, F., E. Marcello, et al. (2009). "Postsynaptic density-membrane associated guanylate kinase proteins (PSD-MAGUKs) and their role in CNS disorders." Neuroscience **158**(1): 324-333.
Gilling, K. E., C. Jatzke, et al. (2009). "Potency, voltage-dependency, agonist concentration-dependency, blocking kinetics and partial untrapping of the uncompetitive N-methyl-D-aspartate (NMDA) channel blocker memantine at human NMDA (GluN1/GluN2A) receptors." Neuropharmacology **56**(5): 866-875.
Gilmour, G. (2009). "Diverse and often opposite behavioural effects of NMDA receptor antagonists in rats: implications for "NMDA antagonist modelling" of schizophrenia." Psychopharmacology (Berl) **205**: 203-216.
Giovannini, M. G., D. Mutolo, et al. (1994). "NMDA receptor antagonists decrease GABA outflow from the septum and increase acetylcholine outflow from the hippocampus: a microdialysis study." J Neurosci **14**(3 Pt 1): 1358-1365.
Girault, J. A. and P. Greengard (2004). "The neurobiology of dopamine signaling." Arch Neurol **61**(5): 641-644.
Giustizieri, M., M. L. Cucchiaroni, et al. (2007). "Memantine inhibits ATP-dependent K+ conductances in dopamine neurons of the rat substantia nigra pars compacta." J Pharmacol Exp Ther **322**(2): 721-729.
Goldman-Rakic, P. S., S. A. Castner, et al. (2004). "Targeting the dopamine D1 receptor in schizophrenia: insights for cognitive dysfunction." Psychopharmacology (Berl) **174**(1): 3-16.
Goldman-Rakic, P. S., E. C. Muly, 3rd, et al. (2000). "D(1) receptors in prefrontal cells and circuits." Brain Res Brain Res Rev **31**(2-3): 295-301.
Golestani, A., H. Ramshini, et al. (2007). "A study on the two binding sites of hexokinase on brain mitochondria." BMC Biochem **8**: 20.
Gottlieb, E., S. M. Armour, et al. (2003). "Mitochondrial membrane potential regulates matrix configuration and cytochrome c release during apoptosis." Cell Death Differ **10**(6): 709-717.
Gottlieb, R. A. (2001). "Mitochondria and apoptosis." Biol Signals Recept **10**(3-4): 147-161.
Grace, M. S. (2007). "Brain and culture: neurobiology, ideology, and social change." Choice: Current Reviews for Academic Libraries **44**(5): 856-856.
Green, S. M. and C. J. Cote (2009). "Ketamine and neurotoxicity: clinical perspectives and implications for emergency medicine." Ann Emerg Med **54**(2): 181-190.
Gunduz-Bruce, H. (2009). "The acute effects of NMDA antagonism: from the rodent to the human brain." Brain Res Rev **60**(2): 279-286.
Hahn, C. G., H. Y. Wang, et al. (2006). "Altered neuregulin 1-erbB4 signaling contributes to NMDA receptor hypofunction in schizophrenia." Nat Med **12**(7): 824-828.
Heresco-Levy, U. (2003). "Glutamatergic neurotransmission modulation and the mechanisms of antipsychotic atypicality." Prog Neuropsychopharmacol Biol Psychiatry **27**(7): 1113-1123.
Hesselink, M. B., B. G. De Boer, et al. (1999). "Brain penetration and in vivo recovery of NMDA receptor antagonists amantadine and memantine: a quantitative microdialysis study." Pharm Res **16**(5): 637-642.

Howes, O. D. and S. Kapur (2009). "The dopamine hypothesis of schizophrenia: version III--the final common pathway." Schizophr Bull **35**(3): 549-562.

Hunt, C. A., L. J. Schenker, et al. (1996). "PSD-95 is associated with the postsynaptic density and not with the presynaptic membrane at forebrain synapses." J Neurosci **16**(4): 1380-1388.

Iasevoli, F., G. Fiore, et al. (2010). "Haloperidol induces higher Homer1a expression than risperidone, olanzapine and sulpiride in striatal sub-regions." Psychiatry Res **177**(1-2): 255-260.

Iasevoli, F., D. Polese, et al. (2007). "Ketamine-related expression of glutamatergic postsynaptic density genes: possible implications in psychosis." Neurosci Lett **416**(1): 1-5.

Iasevoli, F., C. Tomasetti, et al. (2009). "Dopamine receptor subtypes contribution to Homer1a induction: insights into antipsychotic molecular action." Prog Neuropsychopharmacol Biol Psychiatry **33**(5): 813-821.

Iasevoli, F., C. Tomasetti, et al. (2010). "Divergent acute and chronic modulation of glutamatergic postsynaptic density genes expression by the antipsychotics haloperidol and sertindole." Psychopharmacology (Berl) **212**(3): 329-344.

Ikeda, R., Y. Igari, et al. (2011). "Pharmacodynamic interactions between MDMA and concomitants in MDMA tablets on extracellular dopamine and serotonin in the rat brain." Eur J Pharmacol **660**(2-3): 318-325.

Imre, G., A. Salomons, et al. (2006). "Effects of the mGluR2/3 agonist LY379268 on ketamine-evoked behaviours and neurochemical changes in the dentate gyrus of the rat." Pharmacol Biochem Behav **84**(3): 392-399.

Jacobsson, G. and B. Meister (1994). "Hexokinase I messenger RNA in the rat central nervous system." Mol Cell Neurosci **5**(6): 658-677.

Javitt, D. C. (1987). "Negative schizophrenic symptomatology and the PCP (phencyclidine) model of schizophrenia." Hillside J Clin Psychiatry **9**(1): 12-35.

Javitt, D. C. (2010). "Glutamatergic theories of schizophrenia." Isr J Psychiatry Relat Sci **47**(1): 4-16.

Javitt, D. C. and S. R. Zukin (1991). "Recent advances in the phencyclidine model of schizophrenia." Am J Psychiatry **148**(10): 1301-1308.

Jentsch, J. D., J. D. Elsworth, et al. (1998). "Dysregulation of mesoprefrontal dopamine neurons induced by acute and repeated phencyclidine administration in the nonhuman primate: implications for schizophrenia." Adv Pharmacol **42**: 810-814.

Johnson, J. W. and S. E. Kotermanski (2006). "Mechanism of action of memantine." Curr Opin Pharmacol **6**(1): 61-67.

Johnson, S. A., N. T. Luu, et al. (1999). "Synergistic interactions between ampakines and antipsychotic drugs." J Pharmacol Exp Ther **289**(1): 392-397.

Joseph, A., S. Antony, et al. (2008). "Increased glutamate receptor gene expression in the cerebral cortex of insulin induced hypoglycemic and streptozotocin-induced diabetic rats." Neuroscience **156**(2): 298-304.

Kapur, S. (2004). "How antipsychotics become anti-"psychotic"--from dopamine to salience to psychosis." Trends Pharmacol Sci **25**(8): 402-406.

Kapur, S. and D. Mamo (2003). "Half a century of antipsychotics and still a central role for dopamine D2 receptors." Prog Neuropsychopharmacol Biol Psychiatry **27**(7): 1081-1090.

Kargieman, L., N. Santana, et al. (2007). "Antipsychotic drugs reverse the disruption in prefrontal cortex function produced by NMDA receptor blockade with phencyclidine." Proc Natl Acad Sci U S A **104**(37): 14843-14848.

Kegeles, L. S., A. Abi-Dargham, et al. (2000). "Modulation of amphetamine-induced striatal dopamine release by ketamine in humans: implications for schizophrenia." Biol Psychiatry **48**(7): 627-640.
Kegeles, L. S., D. Martinez, et al. (2002). "NMDA antagonist effects on striatal dopamine release: positron emission tomography studies in humans." Synapse **43**(1): 19-29.
Kim, S. H., M. T. Price, et al. (1999). "Excessive cerebrocortical release of acetylcholine induced by NMDA antagonists is reduced by GABAergic and alpha2-adrenergic agonists." Mol Psychiatry **4**(4): 344-352.
Kruse, M. S., J. Premont, et al. (2009). "Interaction of dopamine D1 with NMDA NR1 receptors in rat prefrontal cortex." Eur Neuropsychopharmacol **19**(4): 296-304.
Krystal, J. H., D. C. D'Souza, et al. (2003). "NMDA receptor antagonist effects, cortical glutamatergic function, and schizophrenia: toward a paradigm shift in medication development." Psychopharmacology (Berl) **169**(3-4): 215-233.
Krystal, J. H., L. P. Karper, et al. (1994). "Subanesthetic effects of the noncompetitive NMDA antagonist, ketamine, in humans. Psychotomimetic, perceptual, cognitive, and neuroendocrine responses." Arch Gen Psychiatry **51**(3): 199-214.
Krystal, J. H., E. B. Perry, Jr., et al. (2005). "Comparative and interactive human psychopharmacologic effects of ketamine and amphetamine: implications for glutamatergic and dopaminergic model psychoses and cognitive function." Arch Gen Psychiatry **62**(9): 985-994.
Lahti, A. C., H. H. Holcomb, et al. (1995). "Ketamine activates psychosis and alters limbic blood flow in schizophrenia." Neuroreport **6**(6): 869-872.
Lahti, A. C., B. Koffel, et al. (1995). "Subanesthetic doses of ketamine stimulate psychosis in schizophrenia." Neuropsychopharmacology **13**(1): 9-19.
Lahti, A. C., M. A. Weiler, et al. (2001). "Effects of ketamine in normal and schizophrenic volunteers." Neuropsychopharmacology **25**(4): 455-467.
Lang, P. J., M. M. Bradley, et al. (1998). "Emotional arousal and activation of the visual cortex: an fMRI analysis." Psychophysiology **35**(2): 199-210.
Lang, U. E., I. Puls, et al. (2007). "Molecular mechanisms of schizophrenia." Cell Physiol Biochem **20**(6): 687-702.
Laruelle, M., A. Abi-Dargham, et al. (1996). "Single photon emission computerized tomography imaging of amphetamine-induced dopamine release in drug-free schizophrenic subjects." Proc Natl Acad Sci U S A **93**(17): 9235-9240.
Leino, R. L., D. Z. Gerhart, et al. (1997). "Ultrastructural localization of GLUT 1 and GLUT 3 glucose transporters in rat brain." J Neurosci Res **49**(5): 617-626.
Lewis, M. and P. W. Howie (1987). "Induction of transient hyperprolactinaemia in neonatal rats by direct or maternal treatment with the dopamine receptor blocker, sulpiride." Acta Endocrinol (Copenh) **115**(3): 357-364.
Li, B., N. Devidze, et al. (2009). "NMDA receptor phosphorylation at a site affected in schizophrenia controls synaptic and behavioral plasticity." J Neurosci **29**(38): 11965-11972.
Li, Y. C., G. Liu, et al. (2010). "Dopamine D(1) receptor-mediated enhancement of NMDA receptor trafficking requires rapid PKC-dependent synaptic insertion in the prefrontal neurons." J Neurochem **114**(1): 62-73.
Lieberman, J. A., J. M. Kane, et al. (1987). "Provocative tests with psychostimulant drugs in schizophrenia." Psychopharmacology (Berl) **91**(4): 415-433.
Lin, P. I. and A. R. Shuldiner (2010). "Rethinking the genetic basis for comorbidity of schizophrenia and type 2 diabetes." Schizophr Res **123**(2-3): 234-243.

Link, W., U. Konietzko, et al. (1995). "Somatodendritic expression of an immediate early gene is regulated by synaptic activity." Proc Natl Acad Sci U S A **92**(12): 5734-5738.

Lipska, B. K. and D. R. Weinberger (2000). "To model a psychiatric disorder in animals: schizophrenia as a reality test." Neuropsychopharmacology **23**(3): 223-239.

Lisman, J. E., J. T. Coyle, et al. (2008). "Circuit-based framework for understanding neurotransmitter and risk gene interactions in schizophrenia." Trends Neurosci **31**(5): 234-242.

Liu, F., S. Grauer, et al. (2008). "ADX47273 [S-(4-fluoro-phenyl)-{3-[3-(4-fluoro-phenyl)-[1,2,4]-oxadiazol-5-yl]-piper idin-1-yl}-methanone]: a novel metabotropic glutamate receptor 5-selective positive allosteric modulator with preclinical antipsychotic-like and procognitive activities." J Pharmacol Exp Ther **327**(3): 827-839.

Lodge, D. and N. A. Anis (1982). "Effects of phencyclidine on excitatory amino acid activation of spinal interneurones in the cat." Eur J Pharmacol **77**(2-3): 203-204.

Lyford, G. L., K. Yamagata, et al. (1995). "Arc, a growth factor and activity-regulated gene, encodes a novel cytoskeleton-associated protein that is enriched in neuronal dendrites." Neuron **14**(2): 433-445.

Maddock, R. J. (1999). "The retrosplenial cortex and emotion: new insights from functional neuroimaging of the human brain." Trends Neurosci **22**(7): 310-316.

Maher, F., T. M. Davies-Hill, et al. (1991). "Expression of two glucose transporters, GLUT1 and GLUT3, in cultured cerebellar neurons: Evidence for neuron-specific expression of GLUT3." Mol Cell Neurosci **2**(4): 351-360.

Maher, F., T. M. Davies-Hill, et al. (1996). "Substrate specificity and kinetic parameters of GLUT3 in rat cerebellar granule neurons." Biochem J **315 (Pt 3)**: 827-831.

Malayev, A., T. T. Gibbs, et al. (2002). "Inhibition of the NMDA response by pregnenolone sulphate reveals subtype selective modulation of NMDA receptors by sulphated steroids." Br J Pharmacol **135**(4): 901-909.

Malherbe, P., V. Mutel, et al. (2003). "Identification of critical residues in the amino terminal domain of the human NR2B subunit involved in the RO 25-6981 binding pocket." J Pharmacol Exp Ther **307**(3): 897-905.

Malhotra, A. K., D. A. Pinals, et al. (1997). "Ketamine-induced exacerbation of psychotic symptoms and cognitive impairment in neuroleptic-free schizophrenics." Neuropsychopharmacology **17**(3): 141-150.

Martins-De-Souza, D., E. Dias-Neto, et al. (2010). "Proteome analysis of schizophrenia brain tissue." World J Biol Psychiatry **11**(2): 110-120.

Martins-de-Souza, D., W. F. Gattaz, et al. (2009). "Proteomic analysis of dorsolateral prefrontal cortex indicates the involvement of cytoskeleton, oligodendrocyte, energy metabolism and new potential markers in schizophrenia." J Psychiatr Res **43**(11): 978-986.

McCall, A. L., A. M. Van Bueren, et al. (1994). "Immunohistochemical localization of the neuron-specific glucose transporter (GLUT3) to neuropil in adult rat brain." Brain Res **659**(1-2): 292-297.

McDermott, E. and P. de Silva (2005). "Impaired neuronal glucose uptake in pathogenesis of schizophrenia - can GLUT 1 and GLUT 3 deficits explain imaging, post-mortem and pharmacological findings?" Med Hypotheses **65**(6): 1076-1081.

McEwen, B. S. and L. P. Reagan (2004). "Glucose transporter expression in the central nervous system: relationship to synaptic function." Eur J Pharmacol **490**(1-3): 13-24.
McIntyre, C. K., T. Miyashita, et al. (2005). "Memory-influencing intra-basolateral amygdala drug infusions modulate expression of Arc protein in the hippocampus." Proc Natl Acad Sci U S A **102**(30): 10718-10723.
Meador-Woodruff, J. H., R. E. King, et al. (1996). "Differential regulation of hippocampal AMPA and kainate receptor subunit expression by haloperidol and clozapine." Mol Psychiatry **1**(1): 41-53.
Meisner, F., C. Scheller, et al. (2008). "Memantine upregulates BDNF and prevents dopamine deficits in SIV-infected macaques: a novel pharmacological action of memantine." Neuropsychopharmacology **33**(9): 2228-2236.
Minkeviciene, R., P. Banerjee, et al. (2004). "Memantine improves spatial learning in a transgenic mouse model of Alzheimer's disease." J Pharmacol Exp Ther **311**(2): 677-682.
Miyamoto, S., R. B. Mailman, et al. (2001). "Blunted brain metabolic response to ketamine in mice lacking D(1A) dopamine receptors." Brain Res **894**(2): 167-180.
Moghaddam, B., B. Adams, et al. (1997). "Activation of glutamatergic neurotransmission by ketamine: a novel step in the pathway from NMDA receptor blockade to dopaminergic and cognitive disruptions associated with the prefrontal cortex." J Neurosci **17**(8): 2921-2927.
Mohn, A. R., R. R. Gainetdinov, et al. (1999). "Mice with reduced NMDA receptor expression display behaviors related to schizophrenia." Cell **98**(4): 427-436.
Nai, Q., S. Li, et al. (2010). "Uncoupling the D1-N-methyl-D-aspartate (NMDA) receptor complex promotes NMDA-dependent long-term potentiation and working memory." Biol Psychiatry **67**(3): 246-254.
Narendran, R., W. G. Frankle, et al. (2005). "Altered prefrontal dopaminergic function in chronic recreational ketamine users." Am J Psychiatry **162**(12): 2352-2359.
Neill, J. C., S. Barnes, et al. (2010). "Animal models of cognitive dysfunction and negative symptoms of schizophrenia: focus on NMDA receptor antagonism." Pharmacol Ther **128**(3): 419-432.
Nichols, C. D. and E. Sanders-Bush (2002). "A single dose of lysergic acid diethylamide influences gene expression patterns within the mammalian brain." Neuropsychopharmacology **26**(5): 634-642.
Nishimura, H., F. V. Pallardo, et al. (1993). "Kinetics of GLUT1 and GLUT4 glucose transporters expressed in Xenopus oocytes." J Biol Chem **268**(12): 8514-8520.
Norton, N., H. J. Williams, et al. (2003). "Mutation screening of the Homer gene family and association analysis in schizophrenia." Am J Med Genet B Neuropsychiatr Genet **120B**(1): 18-21.
Okabe, S. (2007). "Molecular anatomy of the postsynaptic density." Mol Cell Neurosci **34**(4): 503-518.
Olney, J. W. and N. B. Farber (1995). "Glutamate receptor dysfunction and schizophrenia." Arch Gen Psychiatry **52**(12): 998-1007.
Olney, J. W., J. Labruyere, et al. (1991). "NMDA antagonist neurotoxicity: mechanism and prevention." Science **254**(5037): 1515-1518.
Olney, J. W., J. W. Newcomer, et al. (1999). "NMDA receptor hypofunction model of schizophrenia." J Psychiatr Res **33**(6): 523-533.

Palfreyman, R. W., A. E. Clark, et al. (1992). "Kinetic resolution of the separate GLUT1 and GLUT4 glucose transport activities in 3T3-L1 cells." Biochem J **284 (Pt 1)**: 275-282.

Parsons, C. G., W. Danysz, et al. (1999). "Memantine is a clinically well tolerated N-methyl-D-aspartate (NMDA) receptor antagonist--a review of preclinical data." Neuropharmacology **38**(6): 735-767.

Parsons, C. G., A. Stoffler, et al. (2007). "Memantine: a NMDA receptor antagonist that improves memory by restoration of homeostasis in the glutamatergic system--too little activation is bad, too much is even worse." Neuropharmacology **53**(6): 699-723.

Pastorino, J. G. and J. B. Hoek (2003). "Hexokinase II: the integration of energy metabolism and control of apoptosis." Curr Med Chem **10**(16): 1535-1551.

Pastorino, J. G. and J. B. Hoek (2008). "Regulation of hexokinase binding to VDAC." J Bioenerg Biomembr **40**(3): 171-182.

Pastorino, J. G., N. Shulga, et al. (2002). "Mitochondrial binding of hexokinase II inhibits Bax-induced cytochrome c release and apoptosis." J Biol Chem **277**(9): 7610-7618.

Peeters, M., J. M. Maloteaux, et al. (2003). "Distinct effects of amantadine and memantine on dopaminergic transmission in the rat striatum." Neurosci Lett **343**(3): 205-209.

Pietersen, C. Y., F. J. Bosker, et al. (2007). "An animal model of emotional blunting in schizophrenia." PLoS One **2**(12): e1360.

Plichta, M. M., A. B. Gerdes, et al. (2011). "Auditory cortex activation is modulated by emotion: a functional near-infrared spectroscopy (fNIRS) study." Neuroimage **55**(3): 1200-1207.

Polakis, P. G. and J. E. Wilson (1985). "An intact hydrophobic N-terminal sequence is critical for binding of rat brain hexokinase to mitochondria." Arch Biochem Biophys **236**(1): 328-337.

Polese, D., A. A. de Serpis, et al. (2002). "Homer 1a gene expression modulation by antipsychotic drugs: involvement of the glutamate metabotropic system and effects of D-cycloserine." Neuropsychopharmacology **27**(6): 906-913.

Prabakaran, S., J. E. Swatton, et al. (2004). "Mitochondrial dysfunction in schizophrenia: evidence for compromised brain metabolism and oxidative stress." Mol Psychiatry **9**(7): 684-697, 643.

Pycock, C. J., R. W. Kerwin, et al. (1980). "Effect of lesion of cortical dopamine terminals on subcortical dopamine receptors in rats." Nature **286**(5768): 74-76.

Qian, B., Y. Sun, et al. (2011). "Epileptiform response of CA1 neurones to convulsant stimulation by cyclothiazide, kainic acid and pentylenetetrazol in anaesthetized rats." Seizure **20**(4): 312-319.

Radwanska, A., W. Debowska, et al. (2010). "Involvement of retrosplenial cortex in classical conditioning." Behav Brain Res **214**(2): 231-239.

Riederer, P., K. W. Lange, et al. (1991). "Glutamate receptor antagonism: neurotoxicity, anti-akinetic effects, and psychosis." J Neural Transm Suppl **34**: 203-210.

Riva, M. A., F. Tascedda, et al. (1997). "Regulation of NMDA receptor subunit messenger RNA levels in the rat brain following acute and chronic exposure to antipsychotic drugs." Brain Res Mol Brain Res **50**(1-2): 136-142.

Robey, R. B. and N. Hay (2006). "Mitochondrial hexokinases, novel mediators of the antiapoptotic effects of growth factors and Akt." Oncogene **25**(34): 4683-4696.

Roncero, I., E. Alvarez, et al. (2000). "Functional glucokinase isoforms are expressed in rat brain." J Neurochem **74**(5): 1848-1857.

Rosi, S., A. Vazdarjanova, et al. (2006). "Memantine protects against LPS-induced neuroinflammation, restores behaviorally-induced gene expression and spatial learning in the rat." Neuroscience **142**(4): 1303-1315.

Sala, C., G. Roussignol, et al. (2005). "Key role of the postsynaptic density scaffold proteins Shank and Homer in the functional architecture of Ca2+ homeostasis at dendritic spines in hippocampal neurons." J Neurosci **25**(18): 4587-4592.

Scatton, B., P. Worms, et al. (1982). "Cortical modulation of striatal function." Brain Res **232**(2): 331-343.

Schmidt, W. J., H. Krahling, et al. (1991). "Antagonism of AP-5-induced sniffing stereotypy links umespirone to atypical antipsychotics." Life Sci **48**(6): 499-505.

Seeman, P., C. Caruso, et al. (2008). "Memantine agonist action at dopamine D2High receptors." Synapse **62**(2): 149-153.

Sharp, F. R., M. Butman, et al. (1994). "Neuronal injury produced by NMDA antagonists can be detected using heat shock proteins and can be blocked with antipsychotics." Psychopharmacol Bull **30**(4): 555-560.

Shearman, E., S. Rossi, et al. (2006). "Changes in cerebral neurotransmitters and metabolites induced by acute donepezil and memantine administrations: a microdialysis study." Brain Res Bull **69**(2): 204-213.

Sheng, M. and C. C. Hoogenraad (2007). "The postsynaptic architecture of excitatory synapses: a more quantitative view." Annu Rev Biochem **76**: 823-847.

Shiraishi-Yamaguchi, Y. and T. Furuichi (2007). "The Homer family proteins." Genome Biol **8**(2): 206.

Simpson, E. H., C. Kellendonk, et al. (2010). "A possible role for the striatum in the pathogenesis of the cognitive symptoms of schizophrenia." Neuron **65**(5): 585-596.

Simpson, I. A., A. Carruthers, et al. (2007). "Supply and demand in cerebral energy metabolism: the role of nutrient transporters." J Cereb Blood Flow Metab **27**(11): 1766-1791.

Simpson, I. A., K. R. Chundu, et al. (1994). "Decreased concentrations of GLUT1 and GLUT3 glucose transporters in the brains of patients with Alzheimer's disease." Ann Neurol **35**(5): 546-551.

Simpson, I. A., D. Dwyer, et al. (2008). "The facilitative glucose transporter GLUT3: 20 years of distinction." Am J Physiol Endocrinol Metab **295**(2): E242-253.

Snyder, S. H. (1976). "The dopamine hypothesis of schizophrenia: focus on the dopamine receptor." Am J Psychiatry **133**(2): 197-202.

Spanagel, R., B. Eilbacher, et al. (1994). "Memantine-induced dopamine release in the prefrontal cortex and striatum of the rat--a pharmacokinetic microdialysis study." Eur J Pharmacol **262**(1-2): 21-26.

Stathakis, D. G., K. B. Hoover, et al. (1997). "Human postsynaptic density-95 (PSD95): location of the gene (DLG4) and possible function in nonneural as well as in neural tissues." Genomics **44**(1): 71-82.

Steward, O. and P. F. Worley (2001). "A cellular mechanism for targeting newly synthesized mRNAs to synaptic sites on dendrites." Proc Natl Acad Sci U S A **98**(13): 7062-7068.

Stone, W. S., S. V. Faraone, et al. (2004). "Evidence for linkage between regulatory enzymes in glycolysis and schizophrenia in a multiplex sample." Am J Med Genet B Neuropsychiatr Genet **127B**(1): 5-10.

Sui, D. and J. E. Wilson (1997). "Structural determinants for the intracellular localization of the isozymes of mammalian hexokinase: intracellular localization of fusion constructs incorporating structural elements from the hexokinase

isozymes and the green fluorescent protein." Arch Biochem Biophys **345**(1): 111-125.
Szumlinski, K. K., P. W. Kalivas, et al. (2006). "Homer proteins: implications for neuropsychiatric disorders." Current Opinion in Neurobiology **16**(3): 251-257.
Szumlinski, K. K., K. D. Lominac, et al. (2005). "Behavioral and neurochemical phenotyping of Homer1 mutant mice: possible relevance to schizophrenia." Genes Brain Behav **4**(5): 273-288.
Tamminga, C. A. (2006). "The neurobiology of cognition in schizophrenia." J Clin Psychiatry **67 Suppl 9**: 9-13; discussion 36-42.
Tiedtke, P. I., C. Bischoff, et al. (1990). "MK-801-induced stereotypy and its antagonism by neuroleptic drugs." J Neural Transm Gen Sect **81**(3): 173-182.
Tiihonen, J., T. Hallikainen, et al. (2003). "Lamotrigine in treatment-resistant schizophrenia: a randomized placebo-controlled crossover trial." Biol Psychiatry **54**(11): 1241-1248.
Tomasetti, C., C. Dell'Aversano, et al. (2007). "Homer splice variants modulation within cortico-subcortical regions by dopamine D2 antagonists, a partial agonist, and an indirect agonist: implication for glutamatergic postsynaptic density in antipsychotics action." Neuroscience **150**(1): 144-158.
Tomasetti, C., C. Dell'Aversano, et al. (2011). "The acute and chronic effects of combined antipsychotic-mood stabilizing treatment on the expression of cortical and striatal postsynaptic density genes." Prog Neuropsychopharmacol Biol Psychiatry **35**(1): 184-197.
VanElzakker, M., R. D. Fevurly, et al. (2008). "Environmental novelty is associated with a selective increase in Fos expression in the output elements of the hippocampal formation and the perirhinal cortex." Learn Mem **15**(12): 899-908.
Vannucci, S. J., E. M. Gibbs, et al. (1997). "Glucose utilization and glucose transporter proteins GLUT-1 and GLUT-3 in brains of diabetic (db/db) mice." Am J Physiol **272**(2 Pt 1): E267-274.
Verma, A. and B. Moghaddam (1996). "NMDA receptor antagonists impair prefrontal cortex function as assessed via spatial delayed alternation performance in rats: modulation by dopamine." J Neurosci **16**(1): 373-379.
Vollenweider, F. X., K. L. Leenders, et al. (1997). "Metabolic hyperfrontality and psychopathology in the ketamine model of psychosis using positron emission tomography (PET) and [18F]fluorodeoxyglucose (FDG)." Eur Neuropsychopharmacol **7**(1): 9-24.
Wasterlain, C. G. and J. W. Chen (2008). "Mechanistic and pharmacologic aspects of status epilepticus and its treatment with new antiepileptic drugs." Epilepsia **49 Suppl 9**: 63-73.
Weinberger, D. R. (1987). "Implications of normal brain development for the pathogenesis of schizophrenia." Arch Gen Psychiatry **44**(7): 660-669.
Williams, K. (1997). "Interactions of polyamines with ion channels." Biochem J **325 (Pt 2)**: 289-297.
Wilson, J. E. (2003). "Isozymes of mammalian hexokinase: structure, subcellular localization and metabolic function." J Exp Biol **206**(Pt 12): 2049-2057.
Winterer, G. and D. R. Weinberger (2004). "Genes, dopamine and cortical signal-to-noise ratio in schizophrenia." Trends Neurosci **27**(11): 683-690.
Woods, D. F. and P. J. Bryant (1993). "ZO-1, DlgA and PSD-95/SAP90: homologous proteins in tight, septate and synaptic cell junctions." Mech Dev **44**(2-3): 85-89.

Xie, G. C. and J. E. Wilson (1988). "Rat brain hexokinase: the hydrophobic N-terminus of the mitochondrially bound enzyme is inserted in the lipid bilayer." Arch Biochem Biophys **267**(2): 803-810.

Yano, H., Y. Seino, et al. (1991). "Tissue distribution and species difference of the brain type glucose transporter (GLUT3)." Biochem Biophys Res Commun **174**(2): 470-477.

Yuan, J. P., K. Kiselyov, et al. (2003). "Homer binds TRPC family channels and is required for gating of TRPC1 by IP3 receptors." Cell **114**(6): 777-789.

Zdanys, K. and R. R. Tampi (2008). "A systematic review of off-label uses of memantine for psychiatric disorders." Prog Neuropsychopharmacol Biol Psychiatry **32**(6): 1362-1374.